The Journal of Laboratory and Clinical Medicine

VOL. 28 DECEMBER, 1943 NO. 15

CLINICAL AND EXPERIMENTAL

THE PRODUCTION OF STAPHYLOCOCCUS ANTITOXIN*

JAMES B. WEAVER, M.D., MARY WHELAN TYLER, M.D., AND DORIS K. SCHUERMAN, A.B., KANSAS CITY, KAN.

THE tissue, or tissues, responsible for the production of staphylococcus antitoxin are not known, but it is generally assumed that such production takes place somewhere in the hemopoietic system. In an effort to throw some light on this problem, the authors have done two types of experiments. First, various components of the hemopoietic system were "blocked out" in experimental animals and the immune reaction of these animals to staphylococcus toxoid as antigen was observed. Second, extracts from various tissues of immunized animals were examined for the presence of antitoxin as indicated by the titer of antihemolysin.

METHODS

White rabbits of five to six pounds each were used in all experiments. Stimulation of antibody production was produced by subcutaneous injection of Lederle's staphylococcus toxoid three times a week, the initial injection being 0.1 c.c., and the dosage increased by 0.1 c.c. at each injection until a total of 1 c.c. was given at one time. The injection period, therefore, covered a little more than three weeks. Titers of antihemolysin were determined twice a week. This procedure was followed in each series of experiments, and in some series total leucocyte and differential counts and hemoglobin determinations were done daily or three times a week as indicated. In most cases the animals were sacrificed at the end of the study period, and sections of bone marrow from typical long and flat bones, spleen, and liver were taken for microscopic study. The results of the study of the microscopic slides will be reported in another paper.

*From the Hixon Research Laboratory of the University of Kansas Medical School.

This work was financed by the Kansas State Board of Health, through funds derived from the U. S. Public Health Service.

Received for publication, May 17, 1943.

CONTROLS

A series of six control animals was run, and, in addition, two controls were studied concurrently with each series of experiments, making a total of nineteen. The results obtained in each individual series were compared against this relatively large number of control animals.

Seventeen of the 19 control rabbits had no initial antibody titer, while two had 0.6 international units of antihemolysin per c.c. before toxoid injections were begun. These rabbits varied somewhat in the length of time required for the response to stimulation, the antibodies appearing in the serum from the eleventh to the twenty-ninth day after the first injection, the average for the whole series being the eighteenth day. The highest concentration of antibodies in the serum was reached at intervals between the eighteenth and the fortieth day, the average being twenty-two.

BENZENE

It has long been known that benzene has a depressant action on the bone marrow, affecting primarily the elements having to do with the formation of the leucocytes. Kracke[1] has shown that benzene given subcutaneously has a marked depressant action on the white blood cells, particularly the granulocytes, and that the erythrocytes are little affected. This is true, except that there is a definite drop in hemoglobin after benzene injections have been continued for some time.

The authors have observed that rabbits have a variable individual resistance to this substance, so that many of them died quickly with a very low leucocyte count, or in an apparently very toxic condition when the white cell count was not at what could be considered a critical level. Autopsy on these animals showed nothing grossly abnormal. The final series consisted of 9 rabbits which survived long enough to receive the full dosage of toxoid. In addition, there were 4 rabbits which received from 0.5 to 0.7 c.c. of toxoid at one time. The general procedure in this series was to inject 1 c.c. of benzene per kilo of body weight subcutaneously daily until the leucocyte count reached 3,000 or less, and then to inject at intervals the amount of benzene necessary to keep the leucocytes at approximately 2,000. When the leucocytes had been sufficiently depressed, toxoid injections were begun. It was not uncommon for the level of the total white cells to reach relatively high levels, probably due to an effort on the part of the bone marrow to overcome the rapid destruction brought about by the benzene.

Of the 9 rabbits in this series, 7 had no initial titer and did not begin to show serum antibodies until twenty to forty-one days after toxoid injections were begun (average, twenty-seven days), and reaching the height in from twenty to forty-eight days (average, twenty-six). Three never did develop a titer. Chart 1 shows four graphs which are typical of the results obtained in this series. Observe the extent of the depression of the leucocytes produced by benzene injections, particularly in the case of Rabbit 12, and the delay in the appearance of antibodies, as well as the small total amount of antihemolysin present. As shown in Chart 2, in which the average curves of serum antihemolysin for both controls and benzene series are plotted, there is a distinct delay in the appearance of antibodies in the benzene series, and the total content

is much lower than that of the control series. Rabbit 12 shows the combined effect of benzene and toxoid very well. This rabbit was studied for somewhat over three months after benzene injections were begun. The leucocyte count was kept at a very low level most of the time, with the occasional elevations mentioned previously. This rabbit had a negative titer for forty-one days after toxoid injections were begun, in spite of the relatively heavy dose of antigen given. In this case the total dose was carried to 1.5 c.c. and an additional 1 c.c. was given at weekly intervals for two injections. It was not until a week following the last injection that a positive titer was demonstrated. The rabbit died with a leucocyte count of 200.

At autopsy these animals showed nothing grossly abnormal except for a very pale bone marrow. Fig. 1 shows bone marrow from normal control rabbits. In contrast, Fig. 2 shows the hypoplastic bone marrow produced by repeated injections of benzene.

CARBON BLOCK

In an effort to block the reticulo-endothelial system, an 8 per cent suspension of Higgins Waterproof India Ink was injected intravenously and intraperitoneally daily in amounts of 20 c.c. by each route. Injections were continued throughout the study. A suitable interval, based partly on the elevation of the total white count but principally on the appearance in the peripheral circulation of considerable numbers of nucleated red cells, was allowed to elapse before toxoid injections were begun. From 200 to 350 c.c. of the suspension were injected, as a rule, before giving toxoid. Cannon et al.[2] have pointed out the importance of continuing the daily injections, particularly intravenously, throughout the period of study and giving the blocking material in sufficiently large amounts to overcome the tendency to stimulation produced by smaller amounts. As much as 1,220 c.c. of the 8 per cent suspension of ink has been given a rabbit during the period of study. Cannon[2] has shown that the amount of fluid is not significant, for he produced no effect in the rabbits to which he gave isotonic saline by the same routes and in the same quantity as his blocking material.

Of the 9 rabbits used in this series, 7 had no initial antihemolysin titer, and the average time of the appearance of antibodies after toxoid injections was twenty-three days (ten to forty). In these, the maximum titer was obtained on an average of twenty-seven days (fourteen to fifty). Chart 3 shows curves for total white cells and antibody content obtained in four typical animals of this series. Note the marked elevation of the total leucocyte count, the delay in appearance, and the low content of antibodies in the serum. Differential counts showed nothing of particular significance except the appearance of large numbers of nucleated red cells and phagocytosis of carbon particles by the polymorphonuclear leucocytes and monocytes. Hemoglobin showed a steady and marked drop as the block was continued. At the end of the experimental period, the hemoglobin content was well below 7 Gm. per cent in all cases.

Chart 2 shows composite curves plotted against the controls. It may be observed that the curve for the blocked animals is much flatter than that for the controls, that the antibodies appeared later and did not rise to so great a quantity. Of the two rabbits which showed an initial titer, one (39) showed a

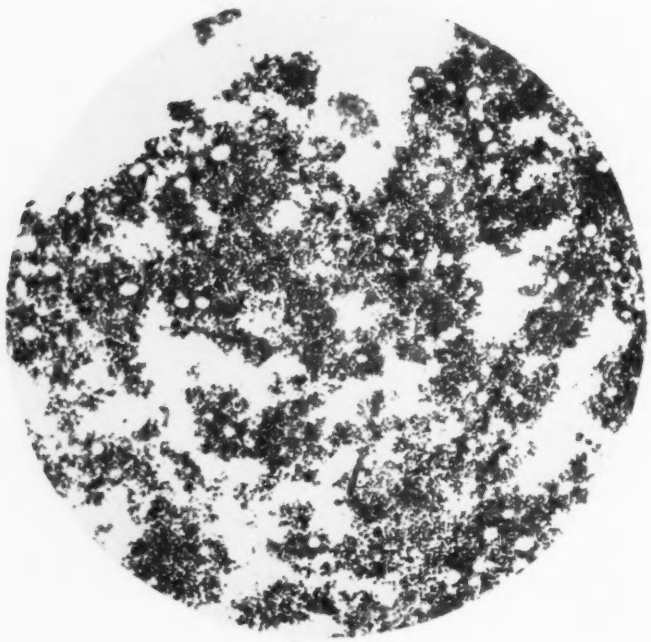

Fig. 1.

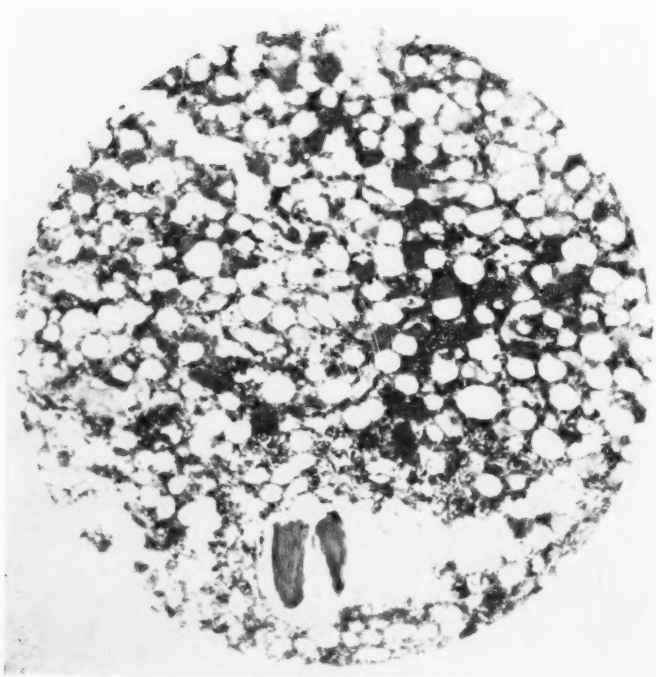

Fig. 2.

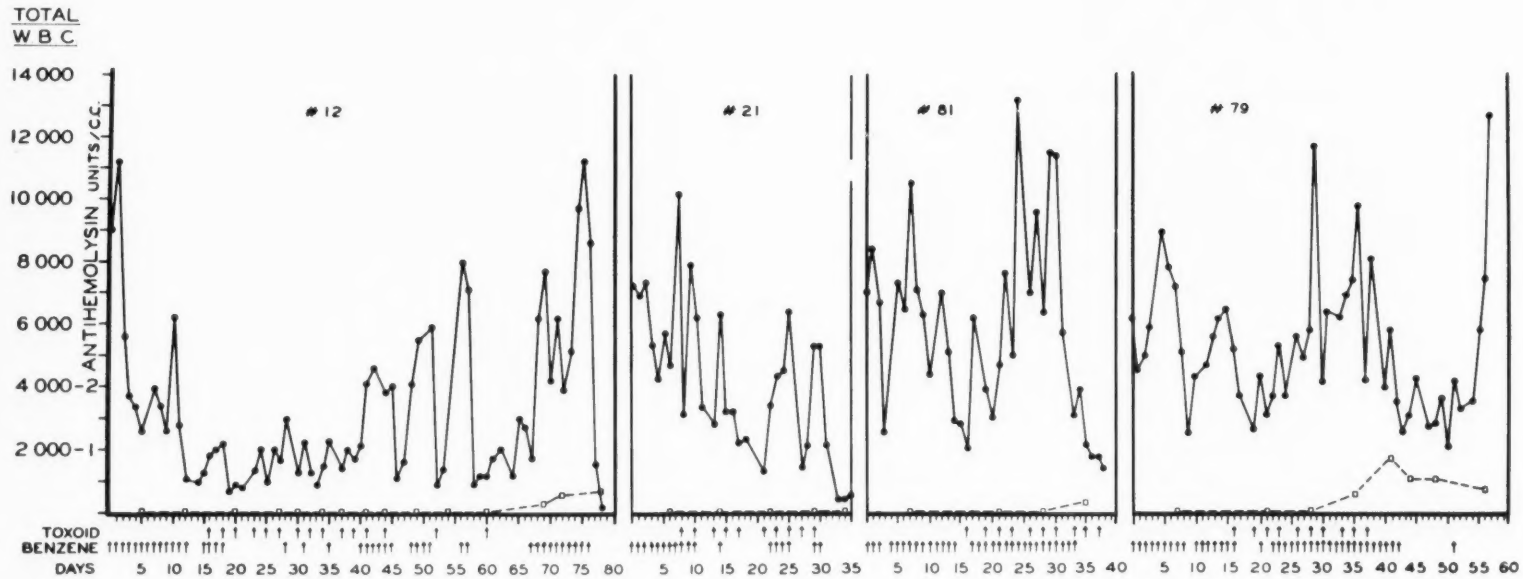

Chart 1.—Total leucocyte count and serum antihemolysin in four typical rabbits in which the bone marrow has been depressed with benzene.

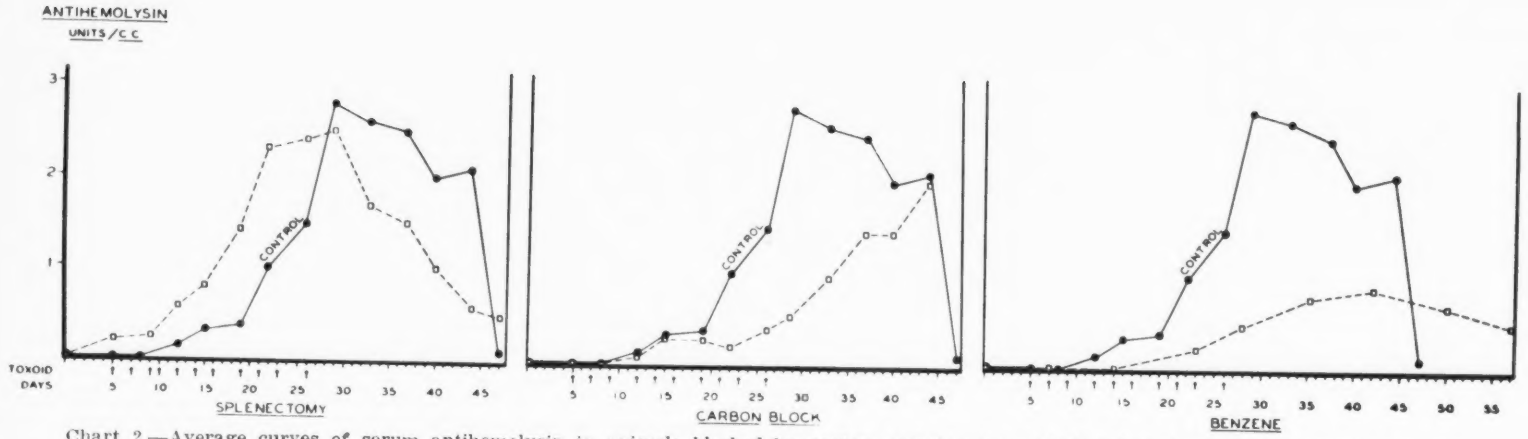

Chart 2.—Average curves of serum antihemolysin in animals blocked by carbon and benzene and in splenectomized and control animals.

diminution in serum antibodies after the ink injections were begun, while the other (42) showed a steady rise.

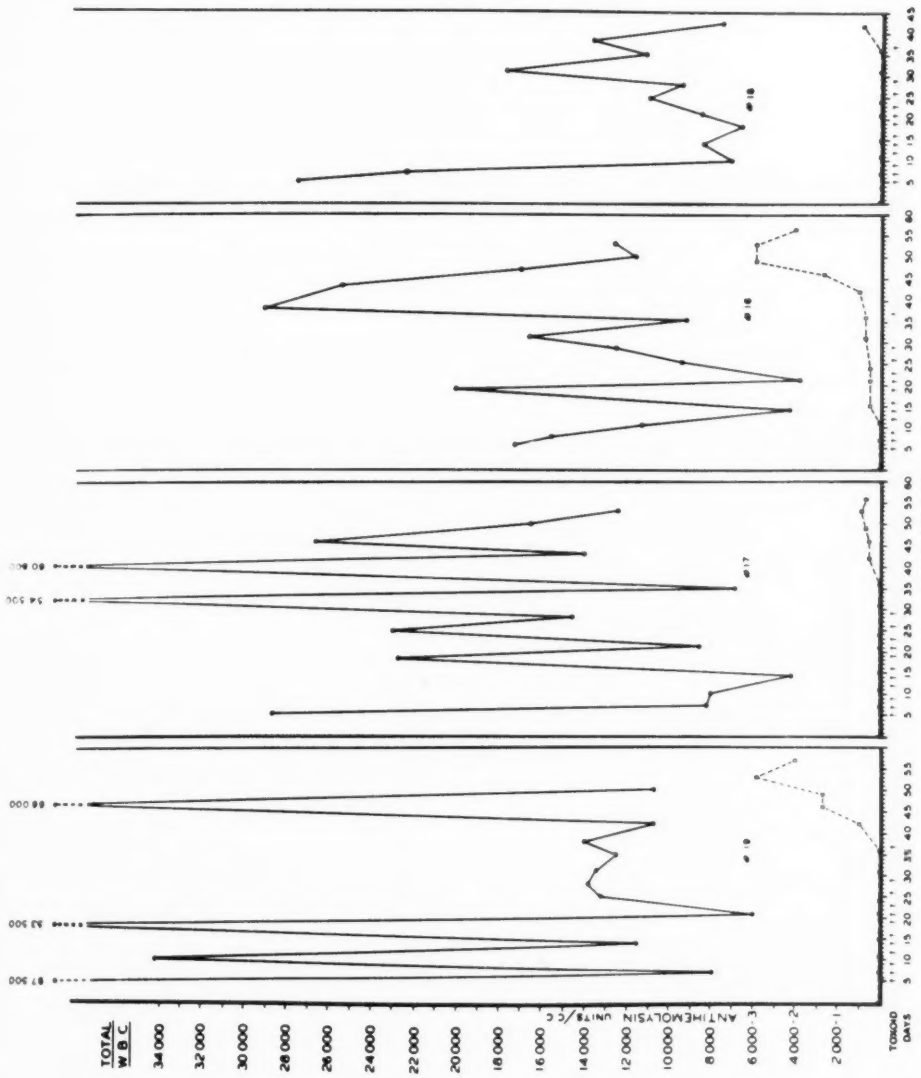

Chart 3.—Total leucocyte count and serum antihemolysin in four typical rabbits in which the hemopoietic system had been blocked with carbon.

The total leucocyte count in the blocked animals was usually relatively high, being well above that of the control animals and increasing to as much as 96,000 in some instances (Chart 3). The animals were killed after a definite decline of serum antibody content occurred, and at autopsy all lymphoid tissue was found to be stained black, the liver and spleen were greatly hypertrophied, the spleen having increased in weight approximately ten times that of the normal animals. The bone marrow was intensely black, the omentum thickened and enlarged. Grossly, the kidneys showed no evidence of having taken up carbon.

SPLENECTOMY

Splenectomy was performed on 11 animals. Three weeks were allowed to elapse following operation before toxoid injections were begun, in order that the

animals might fully recover from the effects of the surgical procedure. Chart 4, where four typical curves are shown, and Chart 2 show the results obtained. It may be noted that the curve for antibody production is similar to that of

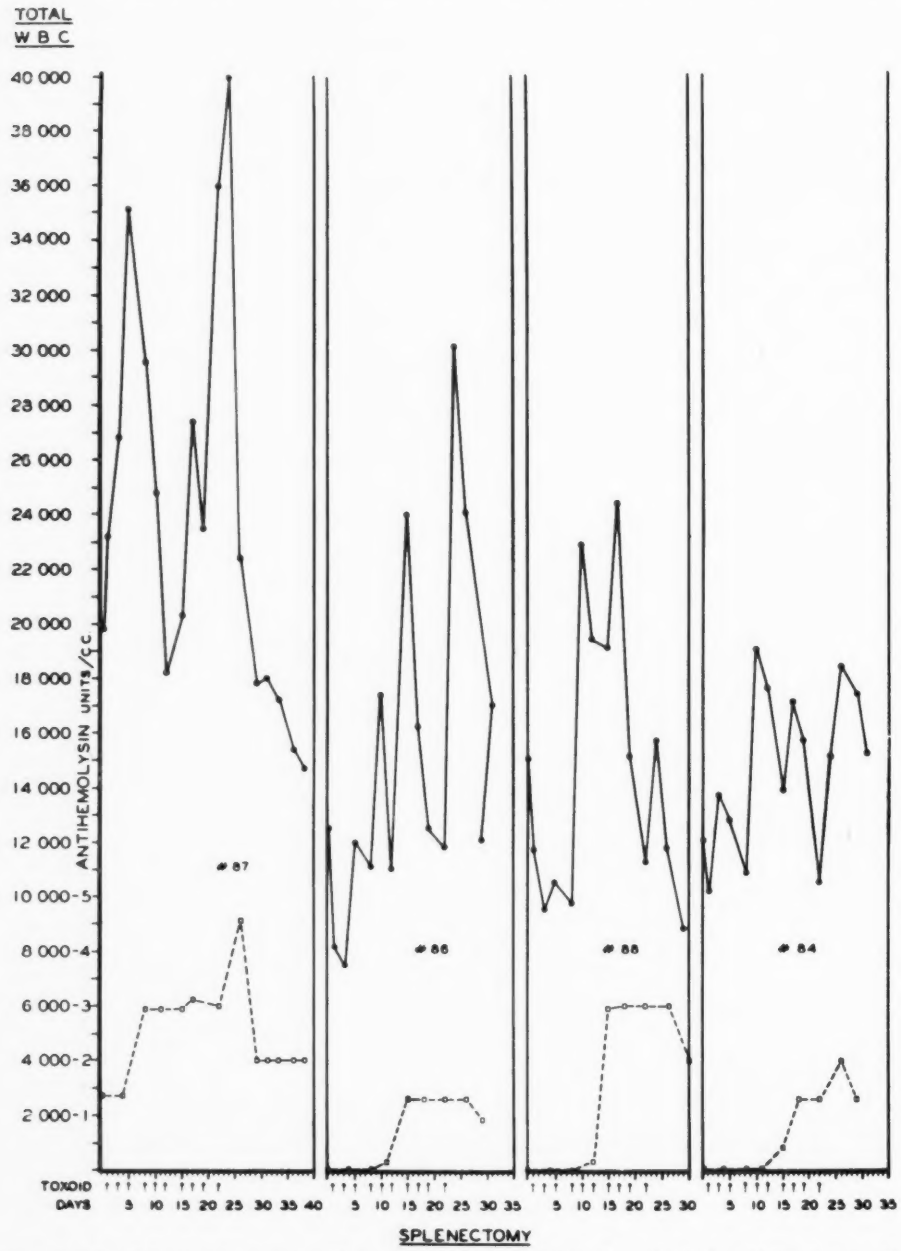

Chart 4.—Total leucocyte count and serum antihemolysin in four typical rabbits from which the spleens had been removed.

the controls in height and shape, differing only in the time of the first appearance of antibodies in the serum. In case of the splenectomy series, antibodies appeared sooner. Analysis of the individual results shows that antibodies appeared

in the serum in two to sixteen days, with an average of eleven days, and the height was reached from the tenth to the twenty-third day (average, nineteen days).

In order to rule out the effect of surgical procedure, nephrectomy was performed on four rabbits. Toxoid injections were begun after a three-week period. The production of antihemolysin showed no particular difference from that in control animals.

Chart 5 is a composite of all curves and shows very clearly the delaying effect of benzene and carbon block on the appearance of antibodies in the serum.

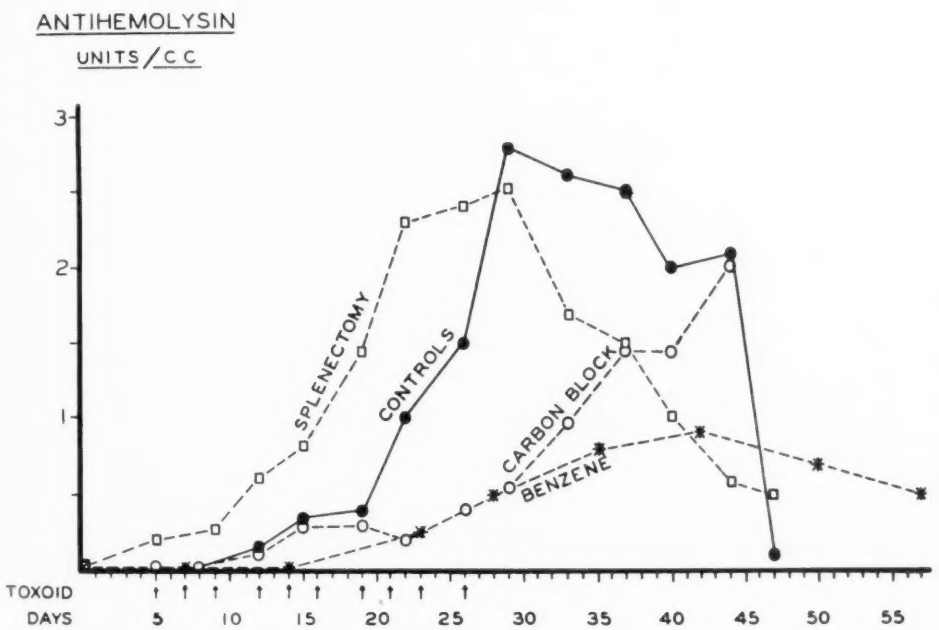

Chart 5.—A composite of all curves showing the depressing effect of carbon and benzene block and the apparent transitory stimulation due to splenectomy.

TISSUE EXTRACTS

It was thought that it might be interesting to study the organs rich in lymphoid tissue for their antibody content. Cary[3] and Motahashi[4] determined tissue antibodies after injecting foreign erythrocytes, by means of extraction with 50 per cent glycerin (14 to 1 dilution) in the incubator for five to six days. This procedure was followed, but we were unable to demonstrate antibodies in the spleen, bone marrow, or liver of sacrificed animals with appreciable known quantities of antitoxin in their blood serum. Undoubtedly this was due to the relatively large dilution recommended. The following procedure was therefore devised.

The animals were exsanguinated and the tissues removed immediately after death, placed in the freezing unit of the refrigerator for twenty-four hours, then pressed through a fine sieve. Two grams of tissue were extracted with 2 c.c. of 0.85 per cent saline for five days in the refrigerator. The tubes were then

centrifuged at high speed, and the supernatant fluid was used for the antihemolysin determinations. The procedure from this point was the same as that used for serum antibody determinations.

By this means we have demonstrated the presence of antihemolysin in bone marrow in 19 of the 56 rabbits in which antibodies were present in varying

TABLE I

ANTIHEMOLYSIN—U/GM. OF TISSUE

RABBIT	SERUM	BONE MARROW	LIVER	SPLEEN	MUSCLE	RBC.
12	0.34	0	0	0	--	--
16	2.98	0	0	0	--	--
17	0.34	0	0	0	--	--
18	0.44	0	0	0	--	--
19	2.98	0	0	0	--	--
21	0	0	0	0	--	--
23	2.98	0	0	0	--	--
24	6.8	0	0	0	--	--
25	0	0	0	0	--	--
31	0	0	0	0	--	--
32	0.34	0	0	0	--	--
36	0	0	0	0	--	--
41	0	0	0	0	--	--
50	0.34	0	0	0	--	--
51	16.8	2.0	0	0	--	0
53	0.26	0	0	0	--	--
62	0.38	0.18	0	--	--	--
64	0.38	0	0	--	--	--
65	0.18	0	0	--	--	--
66	2.0	0.38	0	--	--	--
67	1.34	0	0	0	--	--
68	1.34	0.18	0	0	--	--
69	0.44	0.18	0	0	--	--
79	0.38	0	0	0	0	--
82	0.58	0	0	0	0	--
83	0.38	0	0	0	0	--
84	1.32	0.38	0	--	0	--
87	2.0	0.58	0	--	0	--
88	2.0	1.32	0	0	0	--
115	1.34	0	0	0	0	--
116	2.0	0	0	0	0	--
117	1.34	0	0	0	0	--
132	7.0	0.89	0	0	--	--
152	0.88	0	0	0	0	0
153	0.26	0	0	0	--	0
155	1.32	0	0	0	--	0
157	0.88	0	0	0	--	0
158	1.32	0.34	0	0	--	0
159	1.32	0	0	0	--	0
160	2.98	0	0	0	--	0
161	2.0	0	0	0	--	0
164	2.98	0	0	0	--	0
166	1.32	0	0	0	--	0
167	3.98	1.2	0	0	--	--
168	2.0	1.2	0	0	--	0
169	4.48	2.66	0	0	--	--
170	3.98	1.2	0	0	--	--
171	2.0	0.8	0	0	--	--
172	2.0	0	0	0	--	--
173	3.98	0	0	0.89	--	--
174	4.48	1.2	0	0	--	--
175	1.33	0	0	0	--	0
176	3.98	0.4	0	0	--	0
177	0.6	1.2	0	0.6	--	--
178	0.6	0	0	0	--	--
179	2.0	1.2	0	0	--	0

quantities in the serum. Table I gives the results. Liver, spleen, and bone marrow were studied, and in a few cases, muscle tissue determinations were made as controls. As can be seen in Table I, antibodies were demonstrated only in bone marrow, with the exception of two instances in which small amounts were found in the spleen.

COMMENT

Several factors must be considered in an evaluation of the preceding results. A marked variation in immune response was noted in many of the experimental animals. The cause of this phenomenon is not known, but is usually ascribed to individual characteristics (whatever they may be). The carbon block procedure is not considered to be too efficient. The effect of the carbon was a marked stimulation of leucocyte production instead of depression, and the absorption of carbon by component cells to neutralize their activity, was relied upon to effect the block. It can be readily seen that in this method it is almost impossible to keep the stimulation-block mechanism in complete balance, and it is very easy for the pendulum to swing to the stimulation side.

Benzene is considered a more efficient agent than carbon, but its action is more selective as it apparently affected only the bone marrow. However, its efficiency was somewhat impaired as a result of the toxicity of the drug. When too much benzene was given, the animal died, but if too little was given, bone marrow stimulation ensued.

Splenectomy is considered efficient. The operative procedure might have enhanced antibody production. However, this factor seems to have been ruled out by the results obtained after nephrectomy.

The tissue extract experiments are considered moderately accurate. Care was taken to thoroughly exsanguinate animals so that antibody concentration in the serum would not be measured. The results in Table I are evidence of the success of the method.

It may, therefore, be said that while no definite conclusions may be drawn, yet there is some evidence that staphylococcus antitoxin is produced in the bone marrow.

REFERENCES

1. Kracke, Roy R.: The Experimental Production of Agranulocytosis, Am. J. Clin. Path. **2**: 11, 1932.
2. Cannon, Paul R., Baer, Raymond B., Sullivan, F. L., and Webster, J. R.: The Influence of Blockade of the Reticulo-Endothelial System on the Formation of Antibodies, J. Immunol. **17**: 441, 1929.
3. Cary, W. E.: The Relation of Hemophages to Antibody Production, J. M. Research **43**: 399, 1922.
4. Motahashi, Shinzo: Fixed Tissue Phagocytosis, J. M. Research **43**: 419, 1922.

HUMAN PLASMA AND SERUM TOXICITY

David State, M.D.,* Minneapolis, Minn., and
Milton Levine, M.S., Ph.D.,† Chicago, Ill.

REACTIONS have been reported for both serum and plasma following intravenous administration in humans and animals. The reports are, however, at variance as to the frequency of their occurrence and their severity. Many observers prefer plasma, whereas others by continued use seem to prefer serum for human transfusions. The literature on the subject yields many conflicting observations on the relative merits of the two fluids.

Strumia and his co-workers[1] are firm believers in the value of plasma because of the reactions encountered with serum: "we continue for several reasons[2] to give preference to plasma." McGuiness, Stokes, and Mudd[3] prefer plasma because of the severe reactions observed following the use of lyophile immune serum. Ravdin[4] states, "The frequency with which reactions have occurred following the injection of lyophile serum makes us hesitate to suggest the general acceptance of this material." Meakins[5] is but one of many others reporting serum transfusion reactions.

On the other hand, Levinson and his co-workers[6] continue to use serum and have no doubts as to its safety, "No reactions were observed or need be anticipated if serum is properly prepared." Mellanby[7] urges the use of serum, as do also Self and Scudder.[8] For ease in preparation, the latter prefer serum despite numerous reactions following such transfusions.

It would seem, therefore, that serum is suspected of causing more reactions than plasma, but that some workers prefer serum for other considerations than that of reactions, and these workers either deny that reactions should occur[6] or are of the opinion that the reactions are not important.[8]

The references to plasma reactions are not as copious as for serum. Because of this, many workers have assumed that reactions with plasma do not occur. However, such reactions have been reported. Stephenson and co-workers[9] described a plasma reaction with chills and fever. Self and Scudder[8] observed 4.9 per cent reactions with type specific liquid plasma, 8.2 per cent with pooled liquid plasma, and 5.6 per cent with dried pooled plasma, in a small series of cases. Elliott[10] reported 482 injections of plasma with three reactions. Polayes[11] and Levine and State[12] have given detailed case studies of plasma reactions.

There are many reports of reactions without detailed description as to number or type. Mahoney and his collaborators[13] simply state that, "there have been very few reactions," which indicates that they did observe them. Strumia et al.[14] talk about a "mild urticarial reaction," but conclude that the use of plasma "is simple, safe, and free of reactions."[2]

*Department of Surgery, University of Minnesota.
†Hektoen Institute for Medical Research, Cook County Hospital, and the Department of Bacteriology and Public Health, University of Illinois, Chicago, Illinois.
Received for publication, May 10, 1943.

It is probably correct to conclude that reactions do occur with both serum and plasma. As to serum, early experimentation may explain part of its excessive toxicity. From the time Bowditch[15] first perfused the frog's heart with serum in 1871, much work has been done on serum and plasma transfusion and perfusion in animals. Moldovan[16] in 1910 reviewed the literature up to that period and concluded that serum was more toxic than plasma. He attributed this to a "fibrin ferment" formed in strictly fresh defibrinated blood or blood serum. This ferment was destroyed in about half an hour. For later reviews on the subject, the reader is referred to the papers by Janeway, Richardson, and Park,[17] and Amberson.[18]

During the course of several thousand plasma transfusions at the University of Minnesota Hospitals, we observed reactions frequently enough[19] to invite more detailed observation and experimentation. Since we had been using group specific plasma routinely in the hospital because of ease in preparation, it was possible to make our observations without the complicating factors involved in pooling. In addition, pooling was not practiced routinely, because the main reason for doing so (neutralization of antibodies) had in our opinion never been conclusively established as necessary.

REACTIONS DUE TO A AND B SUBSTANCES

Shortly after the decision was made to study these reactions experimentally, we[12] observed a reaction to plasma in a patient who had been given a routine transfusion on the surgical wards. This patient, G. H., was of blood group O. He developed chills, severe dyspnea, and cyanosis following the intravenous administration of 150 c.c. of undiluted group A plasma. The possibility of incompatible agglutinins in donor plasma was eliminated, since the red cells of the recipient were of group O and hence inagglutinable by the administered plasma. By all theoretical immunologic considerations this group A plasma should have been innocuous. We decided to skin test the patient with the plasma. Previously we[19] had suggested the possible value of such a procedure, knowing well the difficulties of skin tests in general and yet recognizing its invaluable aid in testing for sensitivity to tetanus, pneumococcus, diphtheria, and other antisera.

We found that not only did this patient give a positive skin test with the reacting plasma, but also with three other group A plasmas and one AB plasma, but not with B or O plasmas. The patient was subsequently given 100 c.c. of another group A plasma with resultant chills, cyanosis, and dyspnea. Group O and group B plasmas given intravenously gave no reactions.

From the above and similar cases, we assumed some relationship between the reactions and the blood group of the administered fluid.

Moss[20] in 1910 demonstrated that the hemolytic action of A serum on B cells can be prevented by mixing A serum with B serum. Schiff[21] later found that serum of group A blood contained soluble A substance which seemed to have the same antigenic structure as A substance in the red cells. Levinson and his co-workers[22] found that mixing of A and B sera caused neutralization of the isoagglutinins in both sera. Lubinski[23] attributed this phenomenon to a nonspecific factor, but Aubert and his associates[24] ascribed the neutralization to the dissolved A and B substances found in serum and plasma of the corre-

sponding blood groups and were able to confirm this neutralization by pooling and by the addition of purified A and B substances prepared by Witebsky and his co-workers.[25]

We felt that these dissolved A and B substances might be responsible for the phenomena described. Fortunately we were able to obtain the purified A and B substances from Witebsky, and we used this material to check our hypothesis.

We can summarize our findings as published or still in press. Patients sensitive to the A or B plasma by skin tests with repeated A or B plasmas and some AB plasmas are also sensitive by skin test to the purified substance. These patients are also sensitive to group A or B plasma or AB plasma administered by the intravenous route. They have reactions following the intravenous administration of the purified A and B substances. As control measures, all plasmas are tested for sterility. Some have been divided into two parts, part going to a sensitive individual with a resulting reaction and part to a nonsensitive individual of the same blood group without a reaction. Individuals sensitive to one group specific substance do not necessarily show sensitivity to the other group specific substance, although we have one instance of a patient being sensitive to both.

Patients displaying sensitivity do not necessarily have an allergic history. There does not seem to be any correlation between the amount of antibody present in the recipient and his sensitivity to the factors. However, only those individuals who are in the blood group in which the specific antibody is found corresponding to the specific A or B substance show sensitivity. Not all individuals show this sensitivity even if they have the antibody.

Are the reactions described as being due to the A and B factors important? Are they severe enough to be significant? In most of the cases, the reactions were severe enough to cause great discomfort to the patients. In all our cases we neglected to wait for a natural outcome of the reaction, since we have found that adrenalin relieves the symptoms. It is difficult to say whether any would have proved fatal without the adrenalin. We were not justified in withholding the adrenalin to find out. The severity of any reaction depends upon the reacting material and on the condition of the patient. A reaction which would not disturb a normal individual might kill a patient in a precarious condition.

A case which illustrates the practical importance of the phenomenon follows:

A. J., a woman, age 56, blood group O, had a cholecystectomy for chronic cholecystitis on November 19, 1942. Following the injection of the spinal anesthetic (10 mg. of nupercaine between L_1 and L_2), the blood pressure fell from 170/90 to 100/60. The pulse, however, remained between 70 to 80 per minute. She was given 30 mg. of ephedrine and 0.2 c.c. of neosynephrin intravenously without elevation of the blood pressure. The spinal anesthesia was supplemented with cyclopropane. For the first hour of the operation, the blood pressure remained at about 100/60 and the pulse between 70 to 80 per minute. When the blood pressure failed to rise, the patient was given 500 c.c. of pooled equal parts of A and B plasma intravenously. Ten minutes after the plasma was started, the blood pressure fell from 100/60 to 80/40 and the pulse rose from 80 to 110 per minute. It was then noticed that the patient had developed a generalized giant urticaria. The plasma was stopped, the patient was given 500 c.c. of 5 per cent glucose in saline and 3 c.c. of metrazol intravenously

with elevation of the blood pressure to 110/60 and a fall in the pulse rate to 90 per minute. The skin test to the administered plasma was strongly positive (on the operating table). Subsequently the patient was skin tested and found positive for numerous B plasmas and the purified substance. Skin tests and intravenous administration of type A plasma were without incident.

This case indicates that reactions occur under anesthesia and that they may be serious if unrecognized.

Following the recognition of the factors responsible for some plasma reactions, we were interested in the effect of pooling on the production of these reactions. Although our experience with pooled serum or plasma in cases sensitive to the A and B substances is less extensive than with group specific plasma, we have some results which are indicative. In one case previously reported,[26] the patient was sensitive to both the A and B substances. Skin tests to plasma pooled in the ration of 5:1, 2:1, and 1:1 (A:B plasma) were positive. This confirmed previous observations on other patients.[27] This patient developed a reaction to a transfusion with A plasma but not with O plasma. We then obtained a batch of pooled serum from the Human Serum Center at the University of Minnesota. The pool contained 8 group O, 8 group A, 3 group B, and 1 group AB sera. It gave a positive skin test, and after 10 c.c. had been given intravenously, the patient developed a generalized erythema, epiphora, dyspnea, rapid pulse, and a fall in blood pressure. The same serum pool gave no reaction in a nonsensitive individual.

The effect of pooling on the response to administration of the plasma in sensitive individuals was studied in a case previously reported. In this patient, a reaction occurred following transfusion of a pooled specimen of equal parts of A and B plasma, which also gave a positive skin test. The patient was only sensitive to the B plasma.

It may be that patients sensitive to a single factor may not show as marked a reaction to pooled plasma or serum due to the dilution of the A and B substances. Where the patient is sensitive to both factors, dilution would play less of a role. Neutralization of the A and B factors, in the proportions tried, does not occur in pooled specimens of plasma or serum; hence pooling is not a sure method of preventing reactions due to these substances.

REACTIONS DUE TO FACTORS OTHER THAN THE A AND B SUBSTANCES

We have evidence that reactions may occur because of factors other than the A and B substances. We have reported three cases of such reactions previously.[26] We feel that the following may be the cause of reactions when the A and B substances are not involved:

1. The presence of allergens in the plasma or serum.[28]
2. The presence of reagins in the plasma or serum.[29, 30]
3. The presence of pyrogens.
4. The presence of immunologic factors as yet unknown.

To illustrate transfusion reactions due to factors other than the A and B substances, we present the following cases observed at the University Hospitals in Minneapolis:

Case 1.—F. C., a woman aged 44, of blood group O, had lower and middle lobe lobectomy of the right lung for a bronchial adenoma on 12/11/42. During the operation, she received 400 c.c. of equal parts of A and B plasma and 300 c.c. of A plasma without reaction. On the second post-operative day, five minutes after 200 c.c. of type A plasma had run in, she developed a severe chill lasting twenty minutes with an elevation of temperature from 99 to 101° F. The skin test with the plasma which gave the reaction was positive, but tests with other plasmas, both A and B and the purified A and B substances, were negative. On 12/14/42, she received 200 c.c. of type A plasma without reaction.

Case 2.—V. E., an 18-year-old male, had a right herniorrhaphy on 11/12/42. He was given 500 c.c. of type AB plasma on 11/19/42, 500 c.c. of type B on 11/20/42, 500 c.c. of equal parts of groups A and AB on 11/21/42, 500 c.c. of equal parts of groups A and B on 11/23/42. Approximately one hour after the latter plasma had been given, the patient developed a chill and a fever of 101.6° F. The fever persisted for twelve hours. Skin tests to all plasmas were negative.

Case 3.—D. W., male, aged 44, group O, had a partial colectomy on 11/4/42 and an enterolysis and enterostomy on 11/10/42. During the postoperative period following the second operation, he received 200 c.c. of type O plasma and a similar amount of the same type of O plasma on 11/20/42. On 11/21/42, approximately twenty minutes after receiving 200 c.c. of type O plasma, the patient developed a moderately severe chill and fever of 102° F. The patient was not skin tested with the reaction-producing plasma, but subsequent tests with other group O plasmas gave negative results.

Case 4.—C. G., a 62-year-old male of blood group A had a partial colon resection for carcinoma of the sigmoid colon on 12/14/42. On 12/16/42, he received 200 mg. of equal parts of A and B plasma and 400 c.c. of type A plasma without reactions. On 12/18/42 he developed severe itching and urticaria after receiving 200 c.c. type A plasma. The skin test with this plasma was positive. On 12/19/42, after receiving 200 c.c. of type A plasma, he developed a moderately severe chill and fever of 103° F. The blood pressure fell from 114/70 to 100/70 and the pulse rose from 90 to 120 per minute; the respiration rate increased from 24 to 32 per minute. The skin test to this plasma was also positive. On 12/20/42 and on 12/23/42, he received 200 c.c. of type B plasma without reactions. On each of these occasions, the skin test was negative.

It would be difficult to assign a definite cause to these reactions, but the positive skin test would be more likely to occur associated with an allergic than with a pyrogenic type of reaction. It is certain, however, that these last cases were not due to the A or B factors. We recognize the tendency on the part of clinicians to either overlook reactions or to find them "where they are not." We cite the following case to illustrate a possible false reaction and to demonstrate our awareness of the possibility.

Case 5.—C. C., 70-year-old male, group O, was acutely ill with pyonephrosis. He had daily chills and fever ranging from 100 to 103° F. On 12/10/42 while receiving 200 c.c. of type A plasma, he again developed chills and fever at 103° F. The skin test with the administered plasma was negative. This onset of the chills and fever while plasma was being given was probably coincidental with the daily chills and fever this patient had shown previously. This conclusion was strengthened, although not necessarily proved, by the negative skin test.

CONCLUSION

We have demonstrated what we consider to be important transfusion reactions due to the A and B factors in both plasma and serum. We have also observed other reactions due to factors in plasma other than the A and B substances. We have found the skin test with the plasma a good method of indicating sensitivity to the plasma in many of the cases and an aid in preventing transfusion reactions.

REFERENCES

1. Strumia, M. M., McGraw, J. J., and Reichel, J.: The Preparation and Preservation of Human Plasma, Am. J. Clin. Path. **11**: 175, 1941.
2. Strumia, M. M., Wagner, J. A., Monaghan, J. F.: The Use of Citrated Plasma in the Treatment of Secondary Shock, J. A. M. A. **114**: 1337, 1940.
3. McGuiness, A. C., Stokes, Jr., J. Mudd, S.: Clinical Uses of Human Serums Preserved by the Lyophile Process, J. Clin. Investigation **16**: 185, 1937.
4. Ravdin, I. S.: Report of the Blood Transfusion Association, January, 1941.
5. Meakins, J. C.: Ibid., January, 1941.
6. Levinson, S. O., Rubovits, Jr., F. E., Necheles, H.: Human Serum Transfusions, J. A. M. A. **115**: 1163, 1940.
7. Mellanby, E.: Report of the Blood Transfusion Association, January, 1941.
8. Self, E. B., Scudder, J.: The Therapeutic Use of Serum and Plasma, Liquid and Dried. Blood Substitutes and Blood Transfusions, Springfield, Ill., 1942, Charles C Thomas Company, p. 377.
9. Stephenson, C. S., Cushing, E. H., and Newhouser, L. R.: The Present Status of Blood Substitutes in the United States Navy, Ibid., p. 221.
10. Elliott, J., Busby, G. F., and Tatum, W. L.: Some Factors and Observations on Preparation and Preservation of Dilute Plasma, J. A. M. A. **115**: 1006, 1940.
11. Polayes, S. H., and Squillace, J. A.: Near Fatal Reaction to Transfusion With Dried Human Plasma Solutions, J. A. M. A. **118**: 1050, 1942.
12. Levine, M., and State, D.: A and B Substances as a Cause of Reactions Following Human Plasma Transfusions, J. A. M. A. **120**: 275, 1942.
13. Mahoney, E. B., Kingsley, H. D., Howland, J. E.: The Therapeutic Value of Preserved Blood Plasma. Blood Substitutes, etc., Springfield, Ill., 1942, Charles C Thomas Company, p. 345.
14. Strumia, M. M., McGraw, J. J.: Blood Plasma, J. A. M. A. **118**: 427, 1942.
15. Bowditch, H. P.: Arbeit. a.d. physiol **6**: 139, 1871, Anstalt zu Leipzig.
16. Moldovan, J.: Ueber die Wirkung intravaskularer Injektionen frischen, defibrinierten Blutes, Deutsche med. Wchnschr. **2**: 2422, 1910.
17. Janeway, T. C., Richardson, H. B., and Park, E. A.: Experiments on the Vasoconstrictor Action of Blood Serum, Arch. Int. Med. **21**: 565, 1918.
18. Amberson, W. R.: Blood Substitutes, Biological Reviews of the Cambridge Phil. Soc. **12**: 48, 1937.
19. Levine, M.: Blood Bank Problems, Bull. Staff Meeting, Hospitals of the University of Minnesota **13**: 154, 1942.
20. Moss, W. L.: Studien über Isoagglutinine und Isohemolysine, Folia serolog., 267, 1910.
21. Schiff, F.: Zur Kenntnis blutgruppenspezifischer Antigene and Antikorper, Klin. Wchnschr. **3**: 679, 1924.
22. Levinson, S. O., and Cronheim, A.: Suppression of Iso-Agglutinins and the Significance of This Phenomenon in Serum, J. A. M. A. **114**: 455, 1940.
23. Lubinski, H. H.: The Inhibitory Effect of Human Serum Upon the Iso-Agglutination of Red Cells, J. Immunol. **44**: 95, 1942.
24. Aubert, E. F., Boorman, K. E., Dodd, B. E.: The Agglutinin Inhibiting Substance in Human Serum, J. Path. & Bact. **54**: 89, 1942.
25. Witebsky, E., Klendshoj, N., Swanson, P.: Reduction or Elimination of the Anti A Antibody in O Blood by Means of the Addition of the "A" Specific Substance, J. Infect. Dis. **67**: 188, 1940.
26. Levine, M., and State D.: Plasma and Serum Transfusion Reactions Due to Factors Other Than the A and B Substances. In press.
27. Levine, M., and State D.: Skin Sensitivity to Human Plasma, Science **96**: 68, 1942.
28. Duke, W. W., and Stofer, D. D.: A Severe Case of Allergy Due to Fish Glue, Med. Clin. North America **7**: 1253, 1924.
29. Holder, A. G., and Diefenbach, W. E.: Urticaria—Its Passive Transmission by Blood Transfusion, California and West. Med. **37**: 387, 1932.
30. Garver, W. P.: The Transference of Reagins in Blood Transfusions, J. Allergy **11**: 32, 1939.

THE EFFECT OF SULFONAMIDE COMPOUNDS ON ANTIBODY RESPONSE TO STAPHYLOCOCCUS TOXOID*

JAMES B. WEAVER, M.D., AND MARY WHELAN TYLER, M.D., KANSAS CITY, KAN.

THIS study was undertaken to determine the effect of sulfanilamide and related compounds on the production of antibodies in response to staphylococcus toxoid injections. Only one paper on this subject was found in a review of the literature. Richou and Rastigar[1] found that there is apparently no inhibition of response in antibody development following simultaneous injections of sulfanilamide and staphylococcus anatoxin. They used two groups of five rabbits each. To one group they gave three injections of 0.5 to 3 c.c. of staphylococcus anatoxin at five-day intervals. To the second group they gave anatoxin as to Group 1 plus two intravenous injections at several hour intervals of 3 c.c. of a solution of sulfanilamide containing 1 gram in 150 c.c. saline (0.2 grain at each injection). The dose of the drug was relatively small and the length of the experimental period was short. Increasing the amount of drug given to levels comparable to that given therapeutically and continuing to administer it daily for a sufficiently long period should give a truer picture. Consequently the following plan of procedure was carried out.

White rabbits weighing between five and six pounds each were used. Staphylococcus toxoid and drug injections were begun simultaneously. Toxoid was injected subcutaneously beginning with 0.1 c.c. and increasing by 0.1 c.c. at each injection until 1 c.c. was given at a single injection. Injections were made three times a week. The soluble salts of the drugs (sulfanilamide, sulfapyridine, and sulfathiazole) were used to insure constant dosage, and as nearly as possible complete utilization. Injections were made daily throughout the life of this experiment, a period of eight to nine weeks. An attempt was made to keep the daily dose of the drugs within therapeutic limits, because we were chiefly interested in the effects produced by these drugs in the amounts usually prescribed clinically and not in the effect produced by pushing the dose to toxic levels. A control group of nine rabbits received only toxoid. Antihemolysin titers, total leucocyte counts, and hemoglobin determinations were made twice a week.

NEOPRONTOSIL

Six c.c. of neoprontosil were injected intramuscularly daily to each of seven rabbits, making an average dose of 120 mg. per kilo of body weight, which would be equivalent to 8.4 grams or 140 grains daily to a 70-kilo man. Hemoglobin levels fluctuated slightly but not more than is observed in control

*From the Hixon Laboratories of the University of Kansas School of Medicine and financed by federal funds obtained through the Kansas State Board of Health.

Received for publication, May 17, 1943.

animals living under the same conditions. Total leucocyte counts were not affected, and the antihemolysin response to toxoid injections occurred at approximately the same time after the initial injection and reached a level somewhat higher, on the average, than the controls. (Chart 1.)

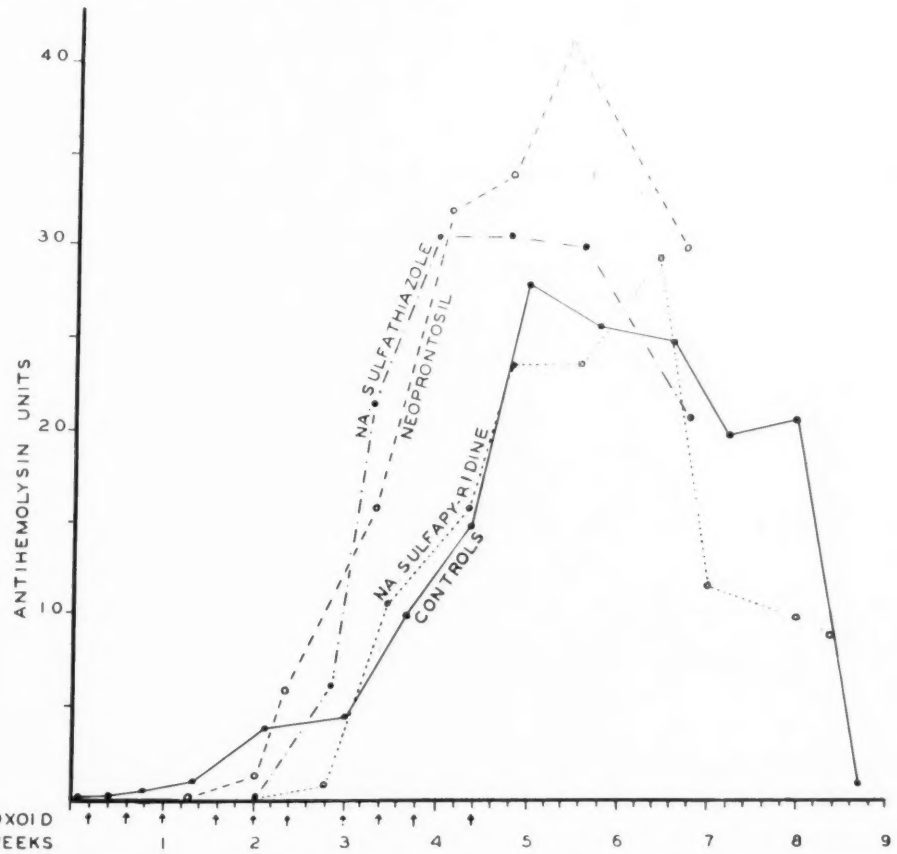

Chart 1.—Composite curves of serum antihemolysin response following staphylococcus toxoid injections in a series of control rabbits and rabbits to which neoprontosil, sodium sulfathiazole and sodium sulfapyradine were given.

SODIUM SULFATHIAZOLE*

Sodium sulfathiazole was made up in 5 per cent solution and given in the amount of 60 mg. per kilo intravenously to six rabbits. The results obtained were similar to those observed during the administration of neoprontosil. No marked fall of hemoglobin concentration was observed, and the total leucocyte count was not markedly reduced, except in two instances when counts of less than 5,000 were obtained. This, however, was only temporary, for normal counts were obtained on the following day, and no subsequent reduction was observed. Chart 1 shows that the average serum antihemolysin curve is similar to the control, except that a slightly more rapid response occurred with a slightly higher level. The concentration of the drug in the blood reached levels of from 1.3 to 5 mg. per 100 c.c.

*Kindly supplied by Dr. Newcomer of Squibbs Institute.

SODIUM SULFAPYRIDINE

A 5 per cent solution of sodium sulfapyridine was used for intravenous injections. Approximately 100 mg. per kilo were given to each of five rabbits daily. Chart 1 shows the antihemolysin curve and its similarity to the controls. The drug reached concentrations of from 1.5 to 3 mg. per 100 c.c. in the blood. Three rabbits were given an average daily injection of 250 mg. per kilo. This was done to see if doubling the therapeutic dose would affect the antibody response in any way. There was no detrimental effect, since the response to toxoid injections was as rapid and the height of the antihemolysin curve was as great as that obtained in the control rabbits. Determinations of sulfapyridine in the blood gave values of 2.5 to 9 mg. per 100 c.c. Hemoglobin concentrations and the total leucocyte counts were not affected by either dosage.

COMMENT

Sodium sulfapyridine, sodium sulfathiazole, and neoprontosil, when given to rabbits in doses which correspond to the average therapeutic dose or higher, have no effect on hemoglobin concentration, leucocyte count, or antibody response to staphylococcus toxoid injections. The antihemolysin titers became positive as soon, and in some cases sooner, in control rabbits and reached correspondingly high levels. In general, the same type of curve was obtained, the fall in titer occurring at approximately the same time after the last toxoid injection.

REFERENCE

Richou, R., and Rastigar, R.: Les injections de sulfamide, faites simultanement a des injections d'anatoxine staphylococcique, ne semblent pas influencer chez le lapin, le developpement de l'antitoxine specifique, Comp. rend. Soc. de Biol. **131**: 855, 1939.

A NOTE ON CYSTS AND ABSCESSES INDUCED IN THE RAT BY THE INJECTION OF OILS*

FREDERICK E. EMERY, PH.D., AND CHARLES S. MATTHEWS, M.A.
BUFFALO, N. Y.

RECENTLY WE showed that peanut oil and sesame oil formed cysts when injected into the muscle of rats. Following this report,[1] several inquiries were received from persons interested in the use of oils as vehicles for hormones and other drugs. Therefore, additional experiments were performed, and in this paper we present data pertaining to the formation of cysts and abscesses following intramuscular injections of mazola, olive, cotton seed, sweet almond, sesame, and peanut oil.

METHODS

The procedure was the same for all oils and was performed by giving a single intramuscular injection of 1.0 c.c. of oil in the hind leg muscles of the rats. These rats were killed from two days to one year after the start of the experiment, and the legs were carefully examined for oil, hemorrhages, durability of cyst walls, and abscesses.

RESULTS

Mazola Oil.—The data show that this oil readily forms cysts. These cysts have firm, somewhat transparent walls and contain clear oil. They persist in the muscles for long periods of time and were found at autopsies performed as long as twelve months after the oil was injected (Fig. 1). Not one abscess has been found in more than fifty experiments with mazola oil (Table I).

TABLE I

AUTOPSY DATA TAKEN AT VARYING PERIODS OF TIME FROM A FEW DAYS TO ONE YEAR AFTER AN INTRAMUSCULAR INJECTION OF 1.0 C.C. OF OIL

NUMBER OF EXPERIMENTS	OILS	CONDITION OF OIL	CONDITION OF CYSTS			
			ABSCESSES	PER CENT	CYST WALL PRESENT	CYST WALL ABSENT
39	Mazola	Often yellow	None	--	37	2
50	Olive	Clear	None	--	48	2
46	Cotton seed	Clear or yellow	8	17.4	40	6
108	Sweet almond	Clear or necrotic	43	39.8	97	11
30	Sesame	Clear	None	--	30	None
35	Peanut	Clear	None	--	33	2

Olive Oil.—Cysts were nearly always present after intramuscular injections of olive oil. The cyst wall was thin and more easily broken than was the case with any other oils studied. Yet even these cysts may remain in the muscles for many months (Fig. 1). Oil taken from the cysts was almost always clear

*From the Department of Physiology University of Buffalo.
Received for publication, May 31, 1943.

and free from necrotic foci. Gross abscesses were not found. These facts give olive oil a high rating for intramuscular injection.

Cotton Seed Oil.—Intramuscular injections of cotton seed oil were usually followed by oil cysts. In six out of forty-six experiments cysts were not found. These negative cases are more frequent than those recorded for mazola and olive oil (Table I). The cyst walls appeared to be firmer than those formed after injection of mazola, olive, and peanut oils. Oil taken from the cysts, at intervals, during the first four months, was clear and showed no evidence of infection. The eight abscesses found (Table I) were all found in autopsies performed five to twelve months after the injections were given. Some of these were necrotic areas buoyed by the oil, while others were firm, yellow, necrotic areas from which oil could not be obtained. Since the abscesses were found only after the lapse of considerable time, it seems likely that the necrosis was not due to the irritation of the oil originally injected, but more probably developed after the oil had changed in composition and, hence, had become more irritating to the tissues. These abscesses were never found in the acute inflammatory condition as described below for sweet almond oil.

Sweet Almond Oil.—Table I shows that cysts were formed in nearly all rats injected intramuscularly with sweet almond oil. The cyst walls were well developed, semiopaque in color, and in about half of the cases so durable that the entire cyst could be removed unbroken from the muscles. In these cases the oil was usually clear. Abscesses in various stages of development were found in nearly half of the rats (Table I). These varied from spots floating in the oil to large inflammatory masses weighing as much as ten grams. When both hind legs were injected on the same day, the bilateral swelling of the legs was so severe that a few of the rats thus treated died within five days. Fig. 1 shows various sizes of the abscesses. Sometimes within a few days following the injection of sweet almond oil, the whole area of the leg was inflammatory, the oil floating in tissue fluid tinted with hemoglobin. Within a week or ten days the swelling subsided, and autopsies showed the oil largely or entirely gone and the capsule containing pus in varying amounts. Thus the picture of sweet almond oil after intramuscular injection is not gratifying.

Sesame Oil.—After intramuscular injections of sesame oil, cysts were readily formed, thus confirming previous reports.[1, 2] Additional experiments carried out during the present investigation serve as controls for the other oils, especially in regard to thickness and toughness of the capsule wall and a tendency for abscess formation. The wall developed around sesame oil is quite tough, and the entire cyst can be dissected readily from adjacent tissues without breaking. This is especially true of cysts that have been in the muscles for several months, but even in earlier stages of development the cysts formed around sesame oil are more durable than any of the other cysts studied. Abscess formation is rare, although flocculent white specks involving part or nearly all of the oil are sometimes seen.

Peanut Oil.—The injection of peanut oil intramuscularly was followed by the formation of cysts. The cyst wall is not as thick or durable as those induced by sesame oil. The oil found in the cysts is fairly abundant in amount and clear. Abscesses have not been seen in any of the rats at autopsy. This

oil seems to irritate the tissues very little and, therefore, compares favorably to mazola and olive oils; so far as we can tell, it differs from sesame oil only in the thickness and durablity of the cyst wall.

DISCUSSION

The results described make one wonder if oil should be injected intramuscularly;* certainly in rats it is obvious that sweet almond oil is very irritating to the tissues, and acute inflammation and even death may result. Cotton seed oil is less irritating, and the injected area as a rule does not become swollen; in only a few rats were abscesses formed (Table I).

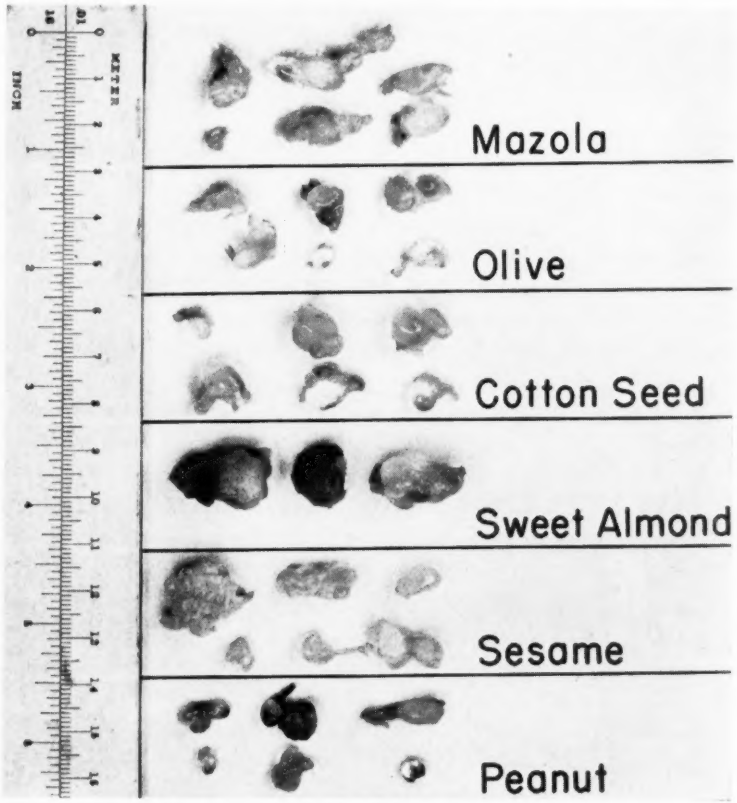

Fig. 1.—Cysts containing oil dissected from the muscles of rats after being in the muscles for varying periods up to one year.

Sesame oil induces thick and durable cystic walls which usually contain clear oil, thus confirming previous reports.[1, 2] These differences place sesame oil in preference to the sweet almond and cotton seed oil for intramuscular injections. As judged by the thickness of the capsule, mazola, olive, and

*Several letters received in regard to our first report contained inquiries concerning oil cysts in humans. Although our data are from rats, the following comments may be of value: Usually autopsy of the leg muscles is not performed. It seems likely that oil cysts would develop, but due to the small size may not be noticed by the patient. Some physicians have told us of complaints by the patient following intramuscular injections of sesame oil. An interne at a local hospital started to give an intramuscular injection of oil, and as a precaution he inserted the needle and aspirated. He withdrew 4 c.c. from an oil cyst formed in the muscles after previous injections. When asked what he did with the oil, he replied, "I injected it all back again."

peanut oils are less irritating than sesame oil; yet it is difficult to place one of these as first choice for intramuscular injection. In our experience all four failed to induce gross inflammatory reactions in the muscles, but like all the other oils used in this study they form oil cysts which remain in the muscles for several months.

When any one of these six oils was injected subcutaneously, the globule readily disseminated, cysts were infrequently seen at autopsy, and abscesses were never found.

Usually the injection was not carried out with antiseptic technique. Since abscesses so readily developed after the injection of sweet almond oil, careful sterilization of the oil, syringe, and needle and application of alcohol to the injected area was followed in many subsequent experiments. This careful technique had no bearing on the incidence of infection.

SUMMARY

Intramuscular injections of mazola, olive, cotton seed, sweet almond, sesame, and peanut oils were given in the hind leg of rats. A tissue capsule developed around the oil within a few days, forming an oil cyst which may remain in the muscles for at least a year. Abscesses developed in nearly half of the rats injected with sweet almond oil and in a few of those injected with cotton seed oil. Sesame oil formed a more durable cyst wall than mazola, olive, and peanut oils, but all four are less irritating than sweet almond and cotton seed oil.

REFERENCES

1. Emery, F. E., Matthews, C. S., and Schwabe, E. L.: The Absorption of Stilbestrol and Theelin From Cysts of Sesame and Peanut Oils, J. Lab. & Clin. Med. **27**: 622, 1942.
2. Deanesly, R., and Parkes, A. S.: Note on Subcutaneous Absorption of Oils by Rats and Mice, With Special Reference to the Assay of Oestrin, J. Physiol. **78**: 155, 1933.

ACUTE DISSECTING AORTIC ANEURYSM: TWO CASES WITH AN ANTE-MORTEM DIAGNOSIS IN ONE*

S. L. ZIMMERMAN, M.D., COLUMBIA, S. C.

DISSECTING aortic aneurysms present a varied clinical syndrome, and it is unusual for the process to present a clear-cut picture suggesting an ante-mortem diagnosis. In addition, the entity is not a common one. Flaxman[1] reports an analysis of 19 cases of dissecting aortic aneurysms which were noted in 14,160 autopsies at the Cook County Hospital between 1929 and 1941, an incidence of 0.14 per cent. In none of these cases was a definite ante-mortem diagnosis reached. He reports a similar incidence at the Charity Hospital of Louisiana, at New Orleans, and the Massachusetts General Hospital.

Up until 1933, 300 cases of dissecting aortic aneurysms were analyzed by Shennan,[2] and in addition 112 more cases have been reported. Prior to 1933 a correct ante-mortem diagnosis was made in only seven cases, and since that time the ante-mortem diagnosis has been made in 25 additional cases, bringing the total to 32 (7.9 per cent) out of 431 reported dissections.[1] In those cases in which an ante-mortem diagnosis was made there were "pointing" clinical findings, such as a sudden onset of a severe, tearing pain, usually thoracic, with variable radiation, collapse, disparity in blood pressure recordings in the two upper extremities, roentgenologic changes, etc. The main pitfall in its correct diagnosis appears to have been the confusion with coronary occlusion. The absence of a typical electrocardiographic evolution of a protracted coronary insufficiency is helpful in those cases which survive the acute dissection. Weiss,[3] however, has reported a case in which the dissection invaded the coronary artery, producing a localized area of myocardial ischemia and typical electrocardiographic changes. In those cases which may be termed "recent" the problem is more difficult. Here the entire gamut of the causes of severe thoracic pain must be differentiated, if pain be a presenting feature. It is well known on the other hand that many dissections are "silent" and are picked up at the necropsy table, much to the surprise of the clinician.

A dissecting aneurysm is a disease of later life, and it is rare before the age of fifty.[4] Flaxman,[1] however, reports one case in a youth twenty-two years of age with hypertensive heart disease.

The exact mode of its production is still a matter of conjecture. It is not definitely known whether an intimal crack or an atheromatous plaque with subsequent medial dissection presents a true picture of its genesis. The other possible mechanism advanced has been the rupture of one or more of the vasa vasorum into an already degenerated media with a subsequent formation of a hematoma. Whether or not the intimal tear results from the rupture of the

*From the Cardiological Service, U. S. Veterans Adm. Facility.

Published with the permission of the Medical Director of the Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

Received for publication, June 2, 1943.

hematoma inwardly or precedes the dissection is not definitely known. The media is usually degenerated, with the production of an inelastic and brittle tube. Cyst formation, idiopathic in origin, with medial necrosis is sometimes found.

Syphilis has often been incriminated as an etiological factor. It is now known that a great many cases show no evidence of syphilis, although the two entities are by no means mutually exclusive. The role that syphilitic mesaortitis plays, in addition to a "nonspecific" medial disease, may enhance the occurrence of a dissection.[4] Hypertension, usually severe, is present in the great majority of cases. It was present in all of the 19 autopsied cases reported by Flaxman.[1] Syphilis was proved in 3, but in conjunction with hypertensive heart disease.

The mode of exitus is usually sudden and dependent upon rupture of the newly formed channel, most often into the pericardial sac, the pleural sacs, or into the peritoneal cavity. Multiple ruptures have been reported. Congestive failure, chronic but progressive, is not an uncommon termination of those cases which are protracted in their courses.

The electrocardiographic changes are often due to a coincident coronary arteriosclerosis; thus, any variety of QRS, ST-T combinations may be seen. If there is a latent coronary insufficiency, the ensuing fall in blood pressure may occasionally result in myocardial ischemia and produce electrocardiographic patterns suggestive of an occlusion. These, however, are usually slighter in degree.[5] If rupture into the pericardial sac results in cardiac tamponade, diagnostic tracings may sometimes be obtained.

The following case is reported as an additional one, unusual in the sense that an ante-mortem diagnosis was made relatively simple by the clinical course of the patient. This largely resulted because the patient was hospitalized during the time of the dissection and was observed at the probable moment of rupture into the abdomen and pericardial sac. The second case is one in which a diagnosis was not made, and it probably is typical of the usual aortic dissection in a patient who is hospitalized shortly before death. It is probable that the dissection had taken place prior to admission and that he had ruptured on the ward.

Case Reports

J. S. H., a fifty-two-year-old white male, was admitted on November 19, 1942, with a diagnosis of hypertension and arteriosclerosis. Past history was not relevant, except that there was a history of syphilis in 1916, followed by three injections of "606." Repeated subsequent serology was negative. He was a known hypertensive, asymptomatic, until four months prior to admission, at which time he noticed exertional dyspnea, progressing to the point of dyspnea at rest and orthopnea. About the same time he first became cognizant of attacks of paroxysmal nocturnal dyspnea, which increased in frequency and severity up to the time of admission. There was no history of anginal syndrome or dependant edema.

Cardiologic examination on November 24 revealed a seriously ill, dyspneic and orthopneic male, slightly cyanotic. The carotid pulsations were exaggerated. The peripheral vessels were sclerotic. Pulses were unequal, the right having a greater amplitude than the left. Blood pressure, right arm, was 220/150; left arm, 180/150. The point of maximum intensity was felt in the fifth intercostal space well outside the midclavicular line. There was some slight increase in retromanubrial dullness. The rhythm was regular. The first sound over the mitral area was of fair quality. No thrills were palpated and no murmurs were heard. Both second sounds over the bases were accentuated. On inspiration, bilateral basal râles were

heard. The liver was not felt; there was no systemic venous engorgement, and no dependent edema was present.

Visualization of the fundi revealed blurring of both discs, many hemorrhages, and hard exudates. The retinal vessels were tortuous, irregular, and fragmented. Wassermann and Kahn tests were negative. Casual urine examinations revealed a trace of albumin and occasional red blood cells. Nonprotein nitrogen was 35 mg. per 100 c.c. Electrocardiogram taken on the day following admission revealed a regular sinus rhythm, with a rate of 95. There were changes indicating left ventricular preponderance; the P-R interval varied from .13 to .16 seconds, with some changes in the configuration of the P waves in succeeding beats. QRS complexes were slurred. The T waves were inverted in 1 and 2 and upright in 3. S-T junction was slightly depressed in the first and second leads. Lead 4-F showed diphasic QRS complexes with an inverted T wave and slight elevation of the S-T junction.

Fig. 1 (Case 1).—Photograph of heart and supracardiac portion of the aorta. Note the linear rent in the supravalvular portion of the aorta. There is no separation of the commissures and no dilatation of the aortic ring. Note the tremendous hypertrophy of the left ventricular musculature. The small markings above the tear are artefacts, due to a hemostatic forceps having been applied.

Interpretation was: 1. Myocardial damage, based on inverted T waves in leads, 1, 2, and 4, depressed ST junction in leads 1 and 2, and slurring of the QRS complexes. 2. Left ventricular preponderance. 3. Wandering pacemaker. X-ray of the chest revealed an enormously increased cardiac shadow with some hypostatic congestion in both bases. The aortic shadow was reported normal. The clinical impression was: 1. Malignant arteriolonephrosclerosis, in impending uremia. 2. Hypertensive and coronary arteriosclerotic heart disease with cardiac enlargement, myocardial damage, and left ventricular failure. 3. Hypertensive neuroretinopathy.

Clinical Course.—The course was entirely afebrile. Respirations varied between 18 and 22; pulse rate ranged between 80 and 100. The patient was digitalized on November 25, 1942, and he showed gradual improvement, so that by November 29 there was no longer evidence of left ventricular failure. At approximately 9 P.M., December 3, he was suddenly taken with severe persistent pain beginning in the region of the suprasternal notch and radiating to the lower chest, abdomen, and down the back, associated with, and followed by, a sensation of numbness in his left leg. Nausea and vomiting were both present. This pain lasted at least three hours, in spite of narcotics. At 9 A.M., December 4, he still complained of numbness in his left leg, and of nausea and vomiting. Examination at that time revealed that he had developed a loud, rough, systolic aortic murmur, which was heard throughout the entire precordium. There was a short, softer, diastolic murmur heard over the aortic area and along the left sternal border. No thrill was palpated. There was no evidence of distention of the neck veins, and the blood pressure was as follows: Right arm, 230/100;

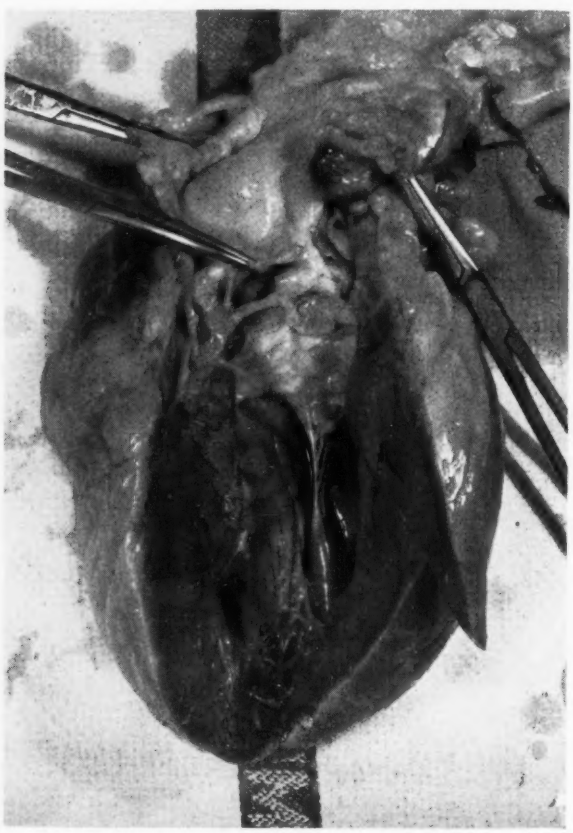

Fig. 2 (Case 1).—Same as depicted in Fig. 1, except that the superior leaf has been elevated to better visualize the tear. See text.

left arm, 150/130. The left dorsalis pedis artery had a markedly diminished pulsation. A tentative diagnosis of acute dissecting aortic aneurysm was made. Beside roentgenogram revealed tremendous aortic and cardiac shadows, but no comparison with the teleoroentgenogram was possible, in view of the differences in technique. It must be stated, however, that the aorta appeared definitely wider than one would expect, were only such differences considered. Electrocardiogram revealed changes in the ST and T contours, the most important of which were the development of upright T waves in the fourth lead and a diphasic T wave in the second lead, as compared to previously inverted T waves. ST junctions were de-

pressed in the first and second leads, and were within normal limits in the fourth lead. The rhythm was regular, rate 150. There were no changes indicative of an acute cardiac tamponade, and there were no changes reported suggesting a diagnosis of acute coronary occlusion. At 4:30 P.M. it was difficult to feel either dorsalis pedis artery. The right posterior tibial artery pulsated normally, but the left could not be felt. He merged into rather rapid congestive failure; pulses were very unequal in amplitude; blood pressure, right arm was 260/110, and could not be obtained by the auscultatory method in the left arm; however, by palpation, the systolic pressure approximated 140 mm. of mercury. At 9 A.M. the following morning he appeared terminal and expired at 9:12. His abdomen became rigid a few minutes prior to his exitus. The final diagnosis was: 1. Malignant arteriolo-nephrosclerosis.

Fig. 3 (Case 1).—Attention is invited to the severe atheromatous changes in the entire thoracic and abdominal aorta. In the lateral view of this it can be seen that the layers have been separated by blood, but the exact point of rupture into the abdominal cavity was not found.

2. Acute dissecting aortic aneurysm, with rupture into the abdomen and pericardial sac. 3. Hypertensive and coronary arteriosclerotic heart disease. Myocardial damage, cardiac enlargement, congestive failure. 4. Hypertensive neuroretinopathy.

POST-MORTEM EXAMINATION, CASE 1

On opening the abdomen, there was a gush of fresh blood which was not clotted, estimated at approximately 750 c.c. The peritoneal surfaces showed little, if any, reaction. On opening the chest, both lungs were found to be normal in appearance. There was no fluid in the pleural sacs. Both lungs had a normal consistency. The right weighed 500 grams, and the left, 600 grams. The cut surface presented no areas of atelectasis or consolidation. The pericardial sac was tensely distended with approximately 500 c.c. of fluid and clotted blood. The serosal surfaces were smooth and glistening. The heart was strikingly enlarged and weighed 800 grams. There was a bluish discoloration of the epicardium posteriorly around the base of the aorta and pulmonary artery. A small rent, 6 mm. in length, extending through the epicardium and the adventitia of the aorta, was noted 2 cm. above the level of the aortic cusps. This could be followed into a cavity separating the adventitia and the media of the aorta.

Fig. 4 (Case 1).—Section through the lower portion of the abdominal aorta. The dark black areas depict clotted blood which has dissected between the media and the adventitia, not quite encircling the aorta.

The wall of the right ventricle was moderately hypertrophied. The bluish discoloration overlying the base of the pulmonary artery was due to a collection of blood, apparently between the media and the adventitia of the pulmonary artery from its origin to a point 3 cm. above the valve cusps. The wall of the left ventricle was markedly hypertrophied, averaging 23 mm. in thickness. The cavity was moderately dilated. The aortic valve cusps were thin and delicate. There was no fusion or widening of the commissures. The ostia of the coronary arteries were patent. The entire aorta was markedly sclerotic, stiff, and inelastic. There was a linear tear in the intima of the aorta at a level 5 mm. above the valve commissures and extending transversely across the right posterior and most of the left posterior valve. This measured 4 cm. in length. The edges were slightly ragged. This tear extended through the intima and media and opened into a complex cavity. This cavity extended, as already described, through a rent in the base of the aorta into the pericardium. It also extended the entire length of the aorta, creating a potential space between the ad-

ventitia and media which in some areas contained clotted blood. It did not completely surround the aorta, but passed about halfway around the circumference at most levels. The arteriosclerosis of the aorta was greatest in the descending portion, the intima of the ascending aorta being almost smooth. There were no tree bark wrinklings of the intima or other luetic stigmata. The exact site of perforation into the abdomen could not be ascertained, but there were no prominent ecchymoses in the periaortic fat at any point. The coronary arteries were diffusely thick-walled, and the lumen was narrowed to about ⅓ of normal. There were no areas of occlusion, however, and there was no dissection of the walls. The myocardium was red-brown and firm, and there were no areas of softening or fibrosis.

MICROSCOPIC EXAMINATION

Heart.—There was a marked hypertrophy of the muscle fibers which had large, irregular, and bizarre nuclei. In a few small areas there was a focal degeneration of occasional muscle fibers. No histologic evidence was seen of inflammatory reaction to the hemopericardium.

Aorta.—The intima was generally thickened. There were several atheromata of typical appearance, varying from the cellular phase to acellular debris with clefts where the cholesterol had been dissolved. The media was divided approximately in the middle by the dissection. In the resulting space was free blood and also adherent platelet-fibrin-leucocyte thrombi. The parallel elastic fibers of the media were intact, except for the two to three fibers immediately adjacent to the dissection where they were occasionally necrotic and often disorganized by the seepage of blood. In a few areas there was a sparse leucocytic infiltration. The line of tear was parallel to the elastic fibers. In the outer layer of the media were occasional small blood vessels and fibrotic scars, both at right angles to and parallel to the elastic lamelae. In the adventitia was a perivascular collection of small round cells, some of moderate magnitude. These inflammatory changes were not diagnostic of a syphilitic meso-aortitis.

Anatomic Diagnosis.—1. Dissecting aneurysm of the aorta with rupture into the abdomen and pericardium. 2. Marked hypertrophy of the left ventricle. 3. Arteriosclerosis of the aorta, severe. 4. Coronary arteriosclerosis. 5. Arteriosclerosis of the kidneys.

CASE NO. 2

E. W. B., a 47-year-old white male, was admitted at 4 P.M., January 13, 1943. In his left lateral chest he had had a severe attack of pain, which radiated to his substernal region on the evening of January 11, 1943. The pain was oppressive in nature and later radiated to the epigastrium; following this he became very weak and broke out into a profuse perspiration. The pain also radiated to both shoulders, but not down either arm. He vomited one and one-half hours later. At time of admission the only residual complaint was some pain in his epigastrium. He was a known hypertensive for many years, but had no complaints referable to his cardiovascular system. There was no history of rheumatic fever or syphilis.

Cardiologic examination, January 14, 1943, revealed a 47-year-old white male, propped up in bed, dyspneic and cyanotic, appearing acutely ill. There was a regular sinus tachycardia. Point of maximal intensity was in the fifth intercostal space at the midclavicular line. The apical impulse, however, was heaving and forceful and gave the impression of a markedly hypertrophied left ventricle. The sounds were booming with an accentuated A-2. There were no thrills palpated and no murmurs heard. There was no evidence of failure. Blood pressure was 240/140. The radial vessels were thickened. Electrocardiogram revealed inverted T waves in the second and third leads and the presence of left axis deviation. There was no evidence of an acute myocardial infarction.

The white blood count was 13,600, with 85 per cent polymorphonuclears and 15 per cent lymphocytes. Nonprotein nitrogen was 35. The urine was not remarkable. Wassermann and Kahn tests were negative. The temperature was 100 degrees.

A diagnosis of hypertensive and coronary arteriosclerotic heart disease, with cardiac enlargement, was made, and in spite of the elevated blood pressure, the probability of a coronary thrombosis was borne in mind. A short time following the above examination the patient expired, quietly and rapidly. Just prior to death the cardiac sounds were not discernible, and the possibility of a ruptured ventricle resulting in an acute cardiac tamponade was considered.

POST-MORTEM EXAMINATION, CASE 2

There was an extravasation of bluish red blood into the retroperitoneal tissues. This extravasation was localized, primarily, on the posterior aspect of the aorta, but also extended down along the right iliac artery almost to the pelvic outlet, around the right lateral surface of the bladder and into the base of the mesentery.

Chest.—There was no fluid nor were there adhesions in the right pleural cavity. In the left pleural cavity there were about 2000 c.c. of fresh blood containing very large fresh clots. The heart was moderately enlarged and weighed 500 Gm. There was an ecchymosis into the adventitia of the pulmonary artery which will be described subsequently. The wall of the left ventricle was markedly thickened, averaging 20 mm. The cavity was moderately dilated. The aortic valve cusps were thin and delicate but contained a few atherosclerotic plaques. There was a moderate arteriosclerosis within the sinuses of Valsalva, which resulted in a moderate fusion at the commissures of all the cusps. The ostia of the coronary arteries were widely patent. The coronary arteries were the seat of widespread and severe arteriosclerosis. There was no marked narrowing of the lumina in the first two centimeters. Beyond the first two centimeters the lumina of all major vessels were markedly narrowed at most levels to a mere pin point. The myocardium was red-brown and firm, and there were no areas of fibrosis noted. The ascending aorta and the arch were elastic, and the intima was almost free of atherosclerosis. Just beyond the opening of the left subclavian artery there was a long oblique linear rent extending through the intima and media. It was 4.5 cm. in length and occurred in a section of the aorta which was free of calcified plaques or other grossly evident abnormalities. From this point down, there was a cavity between the media and adventitia which almost completely encircled the aorta to the level of the origin of the inferior mesenteric artery. At this point, there was another transverse linear rent in the intima 1.5 cm. in length and communicating with the cavity. Below this, the dissection continued down the right side of the aorta and the right iliac artery. The left half of the aorta was not dissected at this level. Within this cavity was a large quantity of freshly clotted blood about 2 cm. in thickness at the level of the upper tear, and diminishing to a few mm. below. The intima of this portion of the aorta contained a considerable number of superficial yellow plaques, a few of which were calcified. There was no tree bark wrinkling of the intima. There was a moderate loss of elasticity of the wall. There was a marked ecchymosis into the adventitia; this was most marked in the thoracic portion of the descending aorta. It also spread upwards, though mild in degree, over the arch of the aorta, on the anterior surface of the aorta and pulmonary arteries to their bases, and into the parietal layer of the pericardium for about 6 cm. from its attachment. There was no dissection between the media and adventitia above the proximal tear. The aorta measured 6 cm. in diameter in the ascending portion and tapered down to 5.5 cm. in the descending portion. There was no constriction at any point or coarctation. The point of rupture of the adventitial tissues into the left pleural cavity could not be determined.

MICROSCOPIC EXAMINATION

Aorta.—The intima was essentially normal. The inner half of the media was composed of the usual parallel lamellae of elastic tissue. The dissection separated the outer quarter from the inner three-quarters of the media. In the space created was an adherent thrombus composed of fibrin and red blood cells with a considerable infiltration of polymorphonuclears. The inner segment of the media subjacent to the thrombus stained poorly, and the nuclei of the elastic and connective tissue fibers appeared to have dropped out. This process affected a considerable length of the media in the section taken and about one-fourth of its thickness. In the necrotic media at the base of the thrombus were many pyknotic nuclei, mostly of polymorphonuclear cells. A similar process affected most of the outer layer of the media at the level of the dissection. This necrotic process was not confined to any one segment of the aorta but was seen at several levels below the dissection. In an elastic-connective tissue stain, the elastic fibers were almost undamaged, but the intercellular substance appeared to have suffered the chief alteration. In the adventitia was a sparse round cell infiltration no more than that normally present.

Comment.—The areas of early necrosis in the aorta probably resulted from an impaired nutrition of the wall secondary to the dissection. This was suggested by their occur-

SIMPSON MEMORIAL INSTITUTE
ANN ARBOR, MICH.

rence beneath the adherent thrombus and not to the same degree elsewhere.

Diagnoses.—1. Dissecting aneurysm of the aorta with rupture into the left pleural sac. 2. Hemothorax, left. 3. Ecchymosis into the posterior mediastinal, retroperitoneal, and pericardial tissues. 4. Arteriosclerosis of the aorta, mild. 5. Infarct, right kidney.

DISCUSSION

These two reported cases, and in addition one other case of dissecting aortic aneurysm discovered at post-mortem examination in 1936, constitute the total number of such cases observed in 31,300 admissions over a period of ten years. During this time there were 1421 deaths and 360 necropsies, giving an incidence of 0.84 per cent of the autopsied material.

The third case referred to was found in a 38-year-old white male, who had a moderate hypertension and died suddenly. The details of his clinical course are not available. Post-mortem examination revealed a tense cardiac tamponade, due to a hemopericardium. The aorta showed two transverse tears 1 cm. above the aortic ring, the edges of which were smooth, as though cut by a knife. The adventitia was separated from the media, forming a second coat which extended from the intimal tears to the diaphragmatic level, encircling the aorta for approximately three-fourths of its circumference. In the descending aorta, 15 cm. from the arch, there were three more transverse intimal tears, which were, also, smooth. There was no evidence of syphilis. Microscopic examination revealed endothelialization of the new tube, a priori evidence that the condition was not very recent. No atherosclerosis of the new aorta was present, as it was in the case recently reported by Graybiel and Sprague.[6] The heart weighed 350 grams. The coronary arteries showed advanced atherosclerotic changes.

In these three cases, hypertension of severe degree was present in two, and of moderate degree in one. The marked variation in the degree of atherosclerosis was striking, being most severe in the case illustrated, and practically absent in the second case. In the third case it was present in moderate degree.

Another interesting point was the fact that the intimal tears in all three cases occurred in a portion of the aorta which was relatively free from atherosclerotic changes. The explanation for this is obscure. The margins of these rents were straight and sharp, all conforming to the type depicted in the illustration. None of the vessels showed any of the acceptable criteria for a diagnosis of syphilitic aortitis, the serology being negative in all three, although a history of inadequately treated lues was obtained from one (Case 1). All three showed some degree of perivascular round cell infiltration in the adventitia, mild in two and moderate in the third (Case 1). There was no dilatation of the aortic ring, however, and there was no separation of the valve commissures.

While, as stated before, the presence of a syphilitic mesaortitis may enhance dissection, Klotz and Simpson[7] state that the luetic process, being a "granulomatous inflammatory process tends to weld the lamellae more closely together,"[8] so that "the wall would split less readily into its anatomic layers."

It is to be noted that the signs which may accompany this syndrome depend, in part, upon the site of the intimal tear, which usually conforms to the level of the origin of the dissection. Its subsequent course is of marked importance and varies to a considerable degree in different cases. These facts are brought out vividly when the two reported cases are compared. In Case 1, the

intimal tear occurred slightly above the aortic ring, the more usual site, resulting in the sudden appearance of a systolic and diastolic murmur. With the progression of the dissection, the likelihood of involvement of the larger vessels of the upper extremity is good, explaining the marked disparity in blood pressure readings obtained in both arms. While the blood pressure was not taken in both arms in the second case, it is doubtful, from the necropsy findings, whether any disparity had existed. Obviously, if the iliacs are involved in the dissection, differences in the pulsations of the arteries of the lower extremities may be apparent. This may or may not accompany a sensation of numbness, tingling, or coldness of the involved extremity, as was present in the first case. It was not looked for in the second case.

The striking findings in these cases were a history of severe thoracic pain, with widespread, variable radiation, the absence of electrocardiographic evidence suggesting a "protracted coronary insufficiency," and the continuation of an unusually marked, pre-existing hypertension. Both patients were obviously ill even after the pain had subsided. In the first case, the actual dissection and rupture were observed while the patient was on the ward. The additional findings of the newly developed murmurs, the disparity in blood pressure readings, and the paraesthesias and coldness of the left leg made the diagnosis relatively obvious.

SUMMARY

1. Two cases of massive dissection of the aorta are presented, in one of which an ante-mortem diagnosis was made. The pathologic description of a third case is given.

2. An idea of the incidence of this entity, and the difficulties in diagnosis are brought out. Certain factors suggesting the diagnosis are discussed.

I am indebted to Dr. Roy Barnett for the pathologic description of the heart and aorta.

REFERENCES

1. Flaxman, N.: Dissecting Aneurysm of Aorta, Am. Heart J. **24**: 654, 1942.
2. Shennan, T.: Dissecting Aneurysm, London, 1934, Medical Research Council.
3. Weiss, S.: Dissecting Aneurysm of the Aorta; Two Cases with Unusual Features, New England J. Med. **218**: 512, 1938.
4. Boyd, Wm.: Textbook of Pathology, Philadelphia 1934, Lea & Febiger.
5. Pardee, Harold E. B.: Clinical Aspects of the Electrocardiogram, New York, 1941, Paul B. Hoeber.
6. Graybiel, Ashton, and Sprague, Howard B.: Dissecting Aneurysm of the Aorta, Am. Heart J. **21**: 530, 1941.
7. Klotz, O., and Simpson, W.: Spontaneous Rupture of the Aorta, Am. J. M. Sc. **184**: 455, 1932.
8. Niehaus, Frederick W., and Wright, Willie D.: Dissecting Aneurysm of the Aorta, J. Lab. & Clin. Med. **26**: 1248, 1941.
9. Pekin, Thomas J., and Nesbitt, John W.: Dissecting Aneurysm of the Aorta, Md. Bull. Vet. Admin. **19**: 96, 1942.
10. Reiss, Jack: Aneurysm of the Aorta; Two Cases, M. Bull. Vet. Admin. **18**: 272, 1942.

THE EXPLANATION FOR THE ABSENCE OF CLOTTING IN BLOODY CEREBROSPINAL FLUID*

M. J. Madonick, M.D.,† and Bernard Newman, Ch.E., M.S.‡
New York, N. Y.

ALTHOUGH textbooks of neurology state that the spinal fluid in cases of subarachnoid bleeding does not clot on standing and that spinal fluid admixed with blood which is produced by trauma during lumbar puncture does clot on standing, very little is reported in the literature regarding the explanation for the lack of clotting in the former.

Fresh bloody cerebrospinal fluids of fifteen patients, varying in age from 20 to 60, were studied. No clotting occurred after the lapse of many hours in any of the specimens. Slight xanthochromia was noted in the supernatant fluid of one of the specimens. Examination of the smeared sediment with Wright's stain revealed the presence of many erythrocytes, normal in size, shape, color, and staining properties, a moderate distribution of leucocytes, and what appeared to be a dearth of thrombocytes.

EXPERIMENTAL PROCEDURE

In five of the fluids selected at random, the calcium concentration varied from 4.3 to 5.1 mg. per 100 c.c. In two of the fluids, the cholesterol was either zero or a slight unreadable trace.

The cerebrospinal fluids were examined both before and after centrifuging for the presence of fibrinogen by adding to each 0.5 c.c. of fluid varying amounts of M/40 $CaCl_2$, from 0.1 to 0.5 c.c., and 0.2 c.c. of thromboplastin suspension made up according to Ziffren et al.[14] In no case did clotting of the cerebrospinal fluid occur.

It was also considered possible that an anticoagulant was present in the spinal fluid which may have accounted for the failure to clot spontaneously. Accordingly, 0.5 c.c. of the fluid was added to 2 c.c. of freshly drawn blood and permitted to stand at room temperature. In all eight of the cases in which this was done, clotting took place. The presence of such a heparin-like anticoagulating factor in the cerebrospinal fluid after nontraumatic subarachnoid hemorrhage may thus be ruled out.

In order to determine whether the amount of blood in the hemorrhagic spinal fluid would not give enough fibrinogen to permit clotting, nontraumatic spinal fluids from three normal individuals in the same age group were colored with freshly drawn blood to about the same intensity as the hemorrhagic fluids

*From the Neuropsychiatric Service of the Morrisania City Hospital, Nathan Savitsky, M.D., Chief, and the Research Laboratory of the Hospital of the Daughters of Jacob, New York City.

†Assistant Visiting Neuropsychiatrist, Morrisania City Hospital, Instructor in Neurology, College of Physicians and Surgeons, Columbia University.

‡Chemist at Hospital of Daughters of Jacob.

Received for publication, June 8, 1943.

and permitted to stand. In all three of these cases, the fluids clotted in about twenty minutes to a jelly-like consistency.

In the next series of studies performed, blood plasma was added in the proportion of 0.5 c.c. to each 2 c.c. of clear centrifuged hemorrhagic spinal fluid to determine whether the criterion for clotting was the presence or absence of fibrinogen. When this was done and 0.2 c.c. of M/40 $CaCl_2$ added, the fluids clotted, but only on addition of a thromboplastin suspension. However, if to the uncentrifuged spinal fluid only plasma and $CaCl_2$ were added, omitting the thromboplastin, clotting also took place. In other words, except for fibrinogen, everything necessary for the clotting of the spinal fluid was present in the fresh hemorrhagic fluid.

The calcium determinations obtained in the fluids tested were within normal limits (Merritt and Bauer[7]). There were no alterations in the cholesterol content (Plaut and Rudy[10]). Lamb[4] emphasized the absence of clot formation in the spinal fluid of meningeal hemorrhage. He performed several experiments with fluids and blood, stating that "even though the amount of blood obtained in spontaneous meningeal hemorrhage be much greater than that required to clot a mixture of normal blood and fluid in vitro, no clotting takes place." He advanced no explanation for this phenomenon.

Our experiments indicate that the lack of clotting is due to the absence of fibrinogen. Normal spinal fluid does not contain this substance (Levinson,[5] Merritt and Fremont-Smith,[8] Strauss and Kaliski[13]). We then have to explain what happens to the fibrinogen of the blood that escapes from the source of the bleeding. We believe that there is clot formation in the cranial cavity and that the fibrinogen is thus removed. The blood that is obtained on lumbar puncture is therefore serum with red blood cells. On autopsy, performed on one of the two fatal cases in our series, clots were found in the subarachnoid spaces. Clots and hematomas in the subarachnoid spaces and even in the ventricles have been frequently described in cases of subarachnoid hemorrhage (Bagley,[1] Fearnsides,[2] Schmidt,[11] Strauss et al.[12]). The explanation for the lack of clotting is the same as that suggested by Neel[9] and by Froin.[3] The former stated that a nonartificial admixture of blood and cerebrospinal fluid "does not coagulate because coagulation has already taken place in the subarachnoid space." However, he gives no data to show how he reached this conclusion. Froin offered the explanation that in subarachnoid hemorrhage the blood forms a clot at and around the site of leaking. He further stated that the proportion of red cells comprising the clot varies according to the severity and the site of the hemorrhage. If the latter be in one of the basal cisterns where the circulation of cerebrospinal fluid is relatively unimpeded, large numbers of erythrocytes are washed off and rapidly diffuse through the subarachnoid space. If on the other hand the site of the bleeding be in the lateral ventricle, the channel of flow is relatively narrow and the great majority of red cells remain trapped in the clot. The red cells that reach the basal cistern then rapidly diffuse through the subarachnoid spaces.

We believe the mechanism is similar to the absence of clotting in menstrual blood (Lozner et al.[6]). Thromboplastin has to be added to the menstrual blood, but its addition does not seem to be necessary for clot formation in the cerebrospinal fluid.

CONCLUSION

Fresh bloody spinal fluids of fifteen patients with meningeal bleeding were examined to determine the cause of the lack of clotting. It was found that fibrinogen was absent in all cases. This, we believe, is due to the occurrence of clotting in and around the brain during which process the fibrinogen is removed from the fluid.

REFERENCES

1. Bagley, C.: Spontaneous Cerebral Hemorrhage, Arch. Neurol. & Psychiat. **27**: 1133, 1927.
2. Fearnsides, E. G.: Intracranial Aneurysms, Brain **39**: 224, 1916.
3. Froin, G.: Les Hemorrhagies Sous-Arachnoïdiennes et Le Mécanisme de l'Hematolyse en général, Facultè de Medecin de Paris, Thèse 1904.
4. Lamb, F. H.: Spontaneous Meningeal Hemorrhage, J. Lab. & Clin. Med. **15**: 531, 1930.
5. Levinson, A.: Cerebrospinal Fluid in Health and in Disease, St. Louis, 1929, C. V. Mosby Co., pp. 61, 89.
6. Lozner, E. L., Taylor, Z. E., and Taylor, F. H. L.: The So-called "Coagulation Defect" in Menstrual Blood, New England J. Med. **226**: 481, 1942.
7. Merritt, H. H., and Bauer, W.: The Equilibrium Between Cerebrospinal Fluid and Blood Plasma. III. The Distribution of Calcium and Phosphorus Between Cerebrospinal fluid and Blood Serum, J. Biol. Chem. **90**: 215, 1931.
8. Merritt, H. H., and Fremont-Smith, F.: The Cerebrospinal Fluid, Philadelphia, 1938, W. B. Saunders Co., p. 30.
9. Neel, A. V.: The Content of Cells and Proteins in the Normal Cerebrospinal Fluid, London, 1939, Oxford University Press, p. 118.
10. Plaut, F., and Rudy, H.: Über die Beziehungen zwischen Blutcholesterin und Liquorcholesterin, Ztschr. f. d. ges. Neurol. u. Psychiat. **146**: 262, 1933.
11. Schmidt, M.: Intracranial Aneurysms, Brain **58**: 489, 1930-31.
12. Strauss, I., Globus, J. H., and Ginsburg, S. W.: Spontaneous Subarachnoid Hemorrhage, Arch. Neurol. & Psychiat. **27**: 1080, 1927.
13. Strauss, I., and Kaliski, D. J.: The Reaction of the Human Cerebrospinal Fluid in Infectious Inflammatory Conditions of the Meninges, A. Research Nerv. & Ment. Dis. **4**: 269, 1924.
14. Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P.: Control of Vitamin K Therapy. Compensatory Mechanisms at Low Prothrombin Levels, Proc. Soc. Exper. Biol. & Med. **40**: 595, 1939.

THROMBOPHLEBITIS MIGRANS*

MORGAN YALE SWIRSKY, M.D., AND CAESAR CASSANO, M.D., NEW YORK, N. Y.

THROMBOPHLEBITIS migrans is a relatively uncommon condition of unknown etiology. It involves chiefly the superficial veins of the upper and lower extremities. Widely separated regions of the body are affected with segments of normal veins between the regions of thrombosis. There is apparently no relation to pre-existing disease of or trauma to the vessels. The veins of the visceral organs are very rarely involved. Tendency to embolize is stated by many observers to be infrequent, while others stress the frequency with which it occurs in the lungs. The disease affects both sexes and all ages. The course is of variable duration and is generally benign, usually terminating in complete recovery. Approximately 100 cases have been reported in medical literature, but in only four acceptable instances has autopsy been performed.[1-4] We *disagree* with Birnberg and Hansen in accepting the case of Hirschhorn et al., because the rheumatic lesion was completely healed, was not more severe than has frequently been found in other autopsies as an incidental observation, and the acute lesion of the heart was nonrheumatic in nature. The present communication is the report of another case of thrombophlebitis migrans with autopsy. The rarity of necropsy observation seems to justify its presentation.

CASE REPORT

The patient, A. C., a 27-year-old white female, was admitted to the medical service of Dr. John Carroll, on March 7, 1942, with a chief complaint of pain and swelling of the right leg and thigh. This was of 1½ weeks' duration. For two weeks prior to the onset, she had been confined to bed with urinary frequency, urgency, burning, and nocturia. Pain which was first noted on the inner aspect of the right ankle joint soon spread up the entire lower limb as high as the groin and was accompanied by swelling of the extremity; chills, fever, and diaphoresis were absent. There was no history of trauma or any previous similar episode.

Past History.—At the age of 6 to 7 years, she had an attack of chorea without polyarthritis or apparent rheumatic heart disease. At the age of 17, shortly after the death of her mother, the patient began to gain weight and soon became very obese. Since the age of 20, she had suffered with attacks of bronchial asthma.

Physical Examination on entry revealed a very obese young white female, appearing somewhat breathless, with dark rings around her eyes. Pupils were round, regular, equal in size, and reacted well to light and accommodation. Oral hygiene was poor and the pharynx was somewhat injected. The neck was supple and no nodes were palpable. The heart was apparently not enlarged, rhythm was regular, tones were of good quality with no audible murmurs, and P_2 was greater than A_2. Blood pressure was 180 systolic and 120 diastolic. Both lung fields were full of asthmatoid wheezes and harsh breath sounds. Numerous white striae were present over the abdomen; palpation was negative. The right lower extremity showed generalized edema and local heat with exquisite tenderness to touch along the course of the saphenous vein. The left lower extremity was not remarkable. There was

*From the First Medical Division and the Pathological Laboratory, City Hospital, Welfare Island, Dept. of Hospitals.

Received for publication, June 11, 1943.

a moderate degree of clubbing of the fingers. No abnormal hirsutism was noted. Temperature was 99.3, pulse, 128, with respirations at 20.

Clinical Course.—Patient was put to bed and treated with palliative measures for three days. The fever rose slowly but steadily during this period to 103.0 accompanied by a rapid pulse rate out of proportion to the temperature, and with increasing dyspnea and cyanosis. The physical findings were suggestive of hypertensive heart disease with left ventricular failure, so-called "cardiac asthma," and a right lower lobe pneumonitis. She was treated with the usual measures with no startling results. A roentgenogram of the chest taken on the third hospital day showed diffuse irregular confluent densities throughout the right lung field, enlargement of the heart with left ventricular predominance, and hepatomegaly. The electrocardiogram revealed a simple tachycardia with a rate of 136, A-V conduction time of 0.16 seconds, slight left axis deviation, diphasic T_1, and inverted Q_3 and T_3. The blood Wassermann reaction was negative, nonprotein nitrogen, 27; sugar, 114; cholesterol, 85; cholesterol esters, 29; icteric index, 15; calcium, 9.7; phosphorus 2.56; albumin, 3.2 per cent; and globulin, 1.9 per cent. The urinalysis revealed a specific gravity of 1.022, two plus albumin, and numerous pus cells. A sputum typing was negative for pneumococci, but showed a *Staphylococcus aureus*. Despite the use of oxygen, digitalis, chemotherapy, and various supportive measures, the patient remained quite uncomfortable and ran an irregular febrile course with accompanying comparable pulse rate. The temperature ranged between 99.2 and 102.6 degrees and the pulse rate between 98 and 130 per minute. A second roentgenogram of the chest taken on the sixth hospital day showed slight clearing of the bronchopneumonic changes.

On the fourteenth hospital day, she suddenly complained of sharp and rather severe pain in the right lower chest. Examination revealed dullness to percussion with scattered moist râles over the right lower lobe, a tender liver enlarged three fingerbreadths below the costal margin, and bilateral ankle edema, more marked on the right side. The chest roentgenogram showed a consolidating process in the right lung field, while the electrocardiogram revealed depressed T_1 and T_2 but otherwise was similar to the previous tracing. Blood and urine cultures were negative although the urine continued to show numerous pus cells, albumin, and a specific gravity of 1.016. The icteric index was 18, nonprotein nitrogen, 35; cholesterol, 143; and cholesterol esters, 40. Red blood cells were 4,400,000; hemoglobin, 70 per cent (Sahli); and white blood cells, 15,000, with 83 per cent polymorphonuclears. Sedimentation time was normal. On the eighteenth day, she complained of sudden onset of severe pain in the posterior aspect of the left thigh and calf with palpatory tenderness along the course of the saphenous vein. During the following twenty-four hours, the usual findings of a thrombophlebitis appeared. She received a course of heparin ("Liquaemin") with good symptomatic relief. The irregular low-grade febrile course and accelerated pulse continued after subsidence of the thrombophlebitis, and episodes of marked dyspnea and some cyanosis continued, accompanied by scattered râles and wheezes in both lung fields and persistently elevated blood pressure of 200/126 to 250/120. The cardiac physical findings were not noteworthy.

On the forty-first day, severe pain in her left upper extremity suddenly occurred. The arm was greatly swollen, hot to touch, exquisitely tender to light palpation, and had a peculiar mottled cyanosis of the skin overlying the involved area. Another course of heparin ("Liquaemin") was given, again with dramatic symptomatic relief, and this thrombophlebitic incident apparently rapidly subsided. On the fifty-first day, she developed signs of a left upper lobe pulmonary infarction which caused her transient discomfort. On the fifty-seventh day, thrombophlebitis of the left external jugular vein developed suddenly, accompanied by severe pain and reappearance of the swelling of the left arm. A blood count the following day showed red blood cells of 3,450,000 and leucocytes of 14,300 with 85 per cent polymorphonuclears. *Staphylococcus aureus* and *Bacillus coli* were isolated from the blood. She gradually lapsed into coma, temperature and pulse rate rose steadily, and the patient expired on the sixty-first day in pulmonary edema. Final temperature was 105, pulse, 142, and respirations were 50.

Autopsy.—(Performed eighteen hours after death. Only the pertinent data are abstracted.) The body is that of a markedly obese white woman of about the stated age. There

is a two-plus pitting edema of the left arm and both legs. The right pupil is slightly larger than the left. The peritoneal cavity contains one liter of clear straw-colored fluid.

Heart.—750 grams. The pericardial sac contains 125 c.c. of a clear straw-colored fluid. The heart is enlarged in all chambers. The right auricle is covered with a thin layer of fibrin. There is abundant epicardial fat. On the anterolateral wall of the right auricle is a firmly attached friable gray thrombus covering the endocardium. The remainder of the mural endocardium is normal. The mitral valve is thickened, and along the line of closure is a row of pinhead-sized firm vegetations similar in color to the body of the cusps. On the auricular surface of the posterior cusp above the line of closure is a single larger vegetation, 1 cm. in length, firmly attached, gray-pink, and somewhat friable. The chordae tendineae are normal. The aortic, tricuspid, and pulmonic valves are normal. The left ventricle is markedly and the right ventricle moderately hypertrophied. The myocardium is of good tone and color and appears free of scar. The coronary arteries have mild to marked patches of atheromatous thickening.

Aorta.—There is an occasional atheromatous plaque in the arch and abdominal portion. The main branches are slightly dilated.

Veins.—The left jugular and innominate veins down to the junction with the superior vena cava contain firmly attached thrombi with reddish cores. Firm gray thrombi occlude the right subclavian and both femoral veins. The venae cavae, portal and hepatic veins are normal.

Lungs.—Right 975 Gm., left 550 Gm. Each pleural sac contains one liter of clear straw-colored fluid and has readily torn stringy fibrous adhesions. The posterior surface of the right lower lobe is covered with fibrin. In the right lung are many areas, irregular in size and shape, firm, granular, and friable; some are gray in color. Near the hilum is a roughly pyramidal area of the lower lobe with the base resting on the horizontal fissure. The center is gray-pink; the periphery, hemorrhagic. In both lungs, many emboli are present in the medium and smaller branches of the arteries. The tracheobronchial tree has copious quantities of seromucus, and the mucosa is mildly congested. The lymph nodes are enlarged, edematous, and blue-black.

Liver.—2,000 Gm. There is an intensification of the markings, giving it a typical nutmeg appearance. The extrahepatic biliary system is negative.

Spleen.—375 Gm. The capsule is tense, irregularly thickened, and has a few stringy fibrous adhesions to the diaphragm. The cut surface has a dark mahogany color and soft consistency. In the subcapsular zone are two small fresh infarcts, one with a gray, the other with a hemorrhagic center. The artery and vein are free of thrombosis.

Urinary System.—Right kidney, 215 Gm.; left kidney, 240 Gm. The fibrous capsules are slightly adherent, leaving a moderately coarse granular surface. The cut surfaces are edematous, but otherwise normal. The lower third of the left ureter is slightly dilated. Otherwise, the system is natural.

G. I. Tract.—There is diffuse submucosal congestion.

Brain.—1,250 Gm. The meninges are markedly engorged. Section fails to reveal any other changes. The pituitary is normal.

Paranasal Sinuses.—The sphenoidal sinus contains a light brown, moderately thick, purulent exudate which flows freely upon opening the sinus. The ethmoids, middle ears, and mastoids are normal.

Pancreas, adrenals, and generative organs are normal.

Histology.—Heart.—The left ventricle has marked hypertrophy; the right, moderate. Small perivascular and interstitial scars are present, most abundant in the interventricular wall and left papillary muscles. In a few there are small foci of lymphocytes, occasionally with mast cells. There are a few isolated foci of polymorphonuclears in the subendocardial zone of the right ventricle. Some of the intrinsic arteries have slight endothelial hyperplasia. The right coronary artery has marked atheroma and dense lymphoid foci in the adventitia. The mitral valve is thickened, fibrotic, and hyaline. It is well vascularized with thin-walled vessels and small thick-walled arteries, having a fine intimal elastification. The small vegetations are dense and fibrotic. The larger vegetation is similar and covered with a platelet thrombus.

Lungs.—There is diffuse capillary engorgement. In the right lung are foci of hemorrhage completely destroying the architectural pattern, surrounded by alveoli containing edema fluid, polymorphonuclears, and hemosiderin-bearing phagocytes. Many of the smaller arteries are occluded by well-organized, partially recanalized thrombi. It both lungs are foci of consolidation surrounding smaller bronchi.

Liver.—There is marked diffuse chronic passive congestion. The cords of the central zone show dissociation, necrobiosis, and bile pigmentation. The portal areas are not remarkable.

Spleen.—There are several small acute infarcts. The remaining portions show moderate interstitial fibrosis, engorged sinusoids, reduction of follicles, and normal central arteries.

Kidneys.—A few of the afferent arterioles have moderately thickened media.

Sphenoid Sinuses.—There is extreme destruction of the architecture of the mucosa with heavy polymorphonuclear, lymphocytic, and plasmacytic infiltration.

Left Internal Jugular Vein.—The lumen is occluded by a thrombus whose peripheral portion is organized and recanalized and has small foci of lymphocytes. The central portion has well-preserved and ghost-like red blood cells. The wall of the vein is edematous and has a mild lymphocytic infiltration.

The other organs show only extreme engorgement.

Anatomical Diagnosis.—Thrombophlebitis migrans; cardiac hypertrophy; healed rheumatic heart disease; recent and old pulmonary emboli; acute bronchopneumonia; generalized chronic passive engorgement; splenomegaly; acute infarcts of spleen; chronic sphenoiditis; obesity.

COMMENT

This case presented many of the well-recognized features of migrating thrombophlebitis and several less common findings. As has been noted frequently, the acute episodes were recurrent in nature and associated with fever. The veins involved were in widely separated regions of the body and not entirely confined to the more superficial ones. As usual, the process appeared first in the subcutaneous veins of one leg and then, by a series of relapses, involved the superficial veins of all the other extremities. Thrombosis of the deep veins, i.e., the femorals, subclavian, innominate, and left external jugular veins, occurred, associated with edema, but without gangrene. No evidence of phlebitis or thrombosis of the visceral[5, 6] or coronary veins[7-10] was noted, although the possibility of these types of involvement has been repeatedly suggested by several authors. Multiple pulmonary and splenic infarcts were present, but no evidence of thrombophlebitis or thrombosis of the pulmonary veins[11, 12] could be detected, so that the only plausible etiology must be embolism from peripheral thrombi. The clinical cardiac findings in this case coupled with those at necropsy had no fundamental relationship to the migrating thrombophlebitis.

The etiology of this disease still remains unsolved, although numerous exhaustive studies have been done on the possibilities of disturbance of clotting mechanism, focal infection, allergic types of sensitivity reaction, and fundamental relationship to Buerger's disease as primary causative factors. Many investigators have taken repeated cultures during the acute phases in the hope of proving a bacteremia or septicemia as an etiologic agent, but the results have been very disappointing. Hedblom[13] reported the presence of "nonmotile spore formers" in the blood stream in his study, and Walker,[14] the finding of *Bacillus alkaligenes*. In our case a blood culture taken early in the course of the disease showed no growth, while a culture taken forty-eight hours before death

showed *Staphylococcus aureus* and *Bacillus coli*. This may have been an agonal phenomenon, and no definite conclusions can be drawn. Unfortunately, cultures from the involved veins were not taken at the autopsy table. The rheumatic lesions found at autopsy appeared to be purely incidental findings having no relationship to the thrombophlebitis. Our conclusions were similar to those adopted in reference to the case of Hirschhorn et al.

The treatment in these cases still leaves much to be desired. Our patient received adequate heparinization, but this treatment failed to prevent further recurrences and thromboses. However, dramatic and rapid symptomatic relief was definitely obtained from the use of this agent.

REFERENCES

1. Lipschitz, M.: Zur der Thrombophlebitis Migrans, Deutsche med. Wchnschr. **55**: 744, 1929.
2. Stern, N. S.: Thrombophlebitis Migrans, With Report of Two Fatal Cases With Autopsies; One Also Showing Primary Carcinoma of the Liver, South. M. J. **27**: 849, 1934.
3. Hirschhorn, L., Lisa, J. R., and Goldstein, N. J.: Thrombophlebitis Migrans; Case Report, Am. Heart J. **17**: 76, 1939.
4. Birnberg, V. J., and Hansen, A. E.: Thrombophlebitis Migrans, J. Pediat. **21**: 775, 1942.
5. Kletz, N.: Thrombo-phlebitis Migrans, The Med. Press and Circular **192**: 371, 1936.
6. Kletz, N.: Thrombo-phlebitis Migrans, Lancet **2**: 938, 1932.
7. Moorehead, T. G., and Abrahamson, L.: Thrombophlebitis Migrans, Brit. M. J. **1**: 586, 1928.
8. Hartfall, S. J., and Armitage, G.: Thrombo-phlebitis Migrans; Report of 2 Cases, Guy's Hosp. Rep. **82**: 424, 1932.
9. Ryle, J. A.: Thrombophlebitis Migrans, Lancet **2**: 731, 1930.
10. Campbell, M., and Morgan, O. G.: Unusual Case of Multiple Thrombosis, Guy's Hosp. Rep. **80**: 34, 1930.
11. Collier, W. T.: Thrombophlebitis Migrans, Involving Deep Veins of All Four Limbs, Lancet **2**: 1408, 1931.
12. Ellison, J. B.: Thrombo-phlebitis Migrans Complicating Scarlet Fever, Brit. J. Child. Dis. **28**: 207, 1931.
13. Hedblom, C. A.: A Case of Thrombophlebitis Migrans, J. A. M. A. **66**: 1777, 1916.
14. Walker, A. B.: Observations on Thrombophlebitis Migrans With Notes of Case, Lancet **2**: 936, 1932.

THE ARTIFICIAL PHOSPHOLIPID MEMBRANE, SEMIPERMEABILITY, AND THE BLOOD-BRAIN BARRIER*

J. H. Weatherby, Ph.D., Richmond, Va.

INTRODUCTION

In a previous paper by the author (1943) it was pointed out that collodion membranes containing lecithin and cephalin exhibit some of the properties of semipermeability generally associated with membranes of living cells. Among those properties found to be common to both artificial and natural membranes may be mentioned the permeability characteristics with respect to weak acids and weak bases and the behavior of the asymmetry potential under various conditions of pH. Weak acids, such as salicylic, permeate the artificial membrane only when pH of the solution permits their existence as such and not as salts; that is to say, permeation occurs from an acid medium in which much of the acid exists as un-ionized molecules, and not from an alkaline medium in which the degree of ionization is very high. Similarly, weak bases, such as nicotine, permeate the artificial membrane only when pH of the solution permits their existence in the molecular form and not as ions; that is, permeation occurs from an alkaline medium and not from an acid one. The explanation proposed for these phenomena is based on ionization of the lecithin or cephalin molecules contained in the membrane, the nature of the ionization depending on pH of the medium in contact with the membrane. This is believed to give rise to the asymmetry potentials observed in membranes, both artificial and natural. Ions bearing charges of similar sign to that of the membrane are repelled by the membrane, and in this manner penetration of the membrane is prevented. Un-ionized molecules are not repelled in this manner, however, and penetration may occur more or less readily. A definite correlation has been found to exist between rate of penetration and concentration of un-ionized molecules of acid or base.

If the above is true, then by making relatively simple changes in molecules it should be possible to change the permeating characteristics of the molecules. For example, derivatives of nicotine in which methyl iodide is added to one or the other, or both the nitrogen atoms contained in the molecule, would be expected to ionize under all conditions of pH which do not actually destroy the molecule, and consequently should not permeate membranes containing lecithin and cephalin under such conditions. Also, the esterification of salicylic acid as in the production of methyl salicylate, or the removal of the carboxyl group to form phenol, should result in permeation of membranes by these substances under all conditions of pH which do not destroy the molecules. Some of the experiments to be described herein are offered as evidence supporting these conclusions.

*From the Department of Pharmacology, Medical College of Virginia.
Received for publication, June 22, 1943.

In a recent review Friedemann (1942) pointed out that many substances, when introduced into the blood stream, pass from blood into brain substance, while certain others do not. The many observations by various authors referred to in this review have resulted in the postulation of the existence of a blood-brain barrier which is highly selective in its ability to permit or deny passage of substances into the brain from the blood. It is interesting to note that among substances which pass from blood to brain are the alkaloids, basic dyes, and certain toxins. Among others which do not readily permeate the brain are the acid dyes, the arsphenamines, a few toxins, and most or all the viruses. The examination of the formulas and physicochemical properties of these various substances, as far as they are known, shows that those which penetrate the brain exist at body pH, at least in part, as un-ionized molecules; whereas, those which do not penetrate exist largely in the ionic forms. Friedemann and Elkeles (1934) found that the ability of toxins to penetrate the brain is closely related to their electrical status. Those which are negatively charged do not penetrate the brain; whereas, those which are positively charged or electrically neutral at body pH do penetrate. Presumably these electrical charges may be due either to ionization of one group or another of the toxin molecule, or to adsorption of ions. An electrical charge would result in either event. The nonpermeation of viruses, as pointed out by Friedemann, may be due to the relatively large sizes of the particles; but it is interesting to note that of those for which such information is available, all are negatively charged. Certain others of the nonpermeating substances, such as trypan blue, congo red, and probably the arsphenamines, are believed to be of colloidal size, and hence do not permeate normal membranes. These facts suggest that the blood-brain barrier may be nothing more nor less than cell membranes containing phospholipids. Obviously, then, any cell membrane containing such substances would be expected to serve as such a barrier. Some of the experiments to be described are believed to have a direct bearing on this question.

EXPERIMENTAL METHODS AND RESULTS

Collodion membranes containing lecithin and cephalin (from brain) were prepared according to methods previously described (Weatherby, 1943) and were used in connection with dialysis cells such as those used previously. An effort was made to include sphingomyelin and cholesterol in some of these membranes, but was abandoned because sphingomyelin is not appreciably soluble in the ether-alcohol solvent used for collodion, and cholesterol crystallizes when the ether content of the solvent is somewhat reduced by evaporation.

Three methyl iodide derivatives of nicotine were tested, as well as nornicotine. Permeability of collodion membranes containing lecithin and cephalin to these nicotine derivatives, as well as various other substances reported either to penetrate the brain or not to penetrate it when injected intravenously, was ascertained by placing solutions of these substances on one side of the membranes and distilled water on the other. The dialysates were examined at intervals for the various substances. Generally two solutions of each substance were used, one at approximately body pH and another that was definitely acid, that is, pH 3.5 to 4.5. The results of these experiments are shown in Table I.

An examination of this table shows that those substances which exist as salts at body pH, and are therefore subject to ionization, do not readily pass through the artificial membrane; whereas, those which exist partly or largely as un-ionized molecules at this pH do penetrate these membranes. The parallelism between penetration or nonpenetration of many of these substances into the brain from the blood stream and the permeation or nonpermeation of these same substances with respect to the artificial membrane is striking indeed. Since most, if not all, cell membranes are believed to contain phospholipids (with some, their presence has been demonstrated conclusively), it seems probable that the blood-brain barrier is one or more membranous systems, which owe their selective properties in a large measure to their phospholipid contents.

TABLE I

SUBSTANCE	pH 3.5-4.5	pH 7.3-7.4	PASSAGE OF BLOOD-BRAIN BARRIER (FRIEDEMANN, 1937)
Nicotine methyl iodide derivatives*	0	0	
Nicotine	0	++	
Nornicotine*	0	+	
Phenol	++	++	
Neoarsphenamine	precipitated	0	
Mapharsen	+	++	
Methylene blue	+	++	+
Toluidine blue	trace	++	
Methyl red†	trace	++	
Neutral red	0	++	+
Eosin	++	+	−
Trypan blue	0	0	−
Congo red	0	0	−

All concentrations 0.25 per cent, except as indicated.
*Molecular equivalent of 0.25 per cent nicotine.
†Saturated.

DISCUSSION

Several of the experiments reported in the table deserve discussion in greater detail. Trypan blue and congo red are colloidal, and hence could not be expected to permeate any but the most highly permeable membranes, although it is well known that the former readily passes through the damaged capillaries in an inflamed area. One would expect that neoarsphenamine, which exists as a salt in an alkaline medium, would be changed into the less highly ionizable acid form in an acid medium and that permeation of the artificial membrane would occur under these conditions. However, precipitation of neoarsphenamine (probably following decomposition) occurred within a few hours after acidification of the solution, and no permeation occurred within twenty-four hours. Methyl salicylate would be the substance to be preferred instead of phenol for comparison with salicylic acid, but this ester is not sufficiently soluble in water for such an experiment. None of the three methyl iodide derivatives of nicotine were found to permeate the artificial membrane.

With nicotine and nornicotine, just as with all other organic bases, the ratio between free base and salt at any given pH may be obtained by means of the Henderson-Hasselbalch equation, $\text{pH} = \text{pK} + \log \frac{\text{Base}}{\text{Salt}}$. The reciprocal

of this relationship holds for weak acids. The value of pK for nicotine is approximately 8.07; that is, half is free base and half salt at this pH. With nornicotine the value of pK is 9.09. At pH 7.4 approximately 18 per cent of the nicotine exists as free base, while at the same pH only about 2 per cent of nornicotine exists as such. Nornicotine permeated the artificial membrane at body pH, but much more slowly than nicotine under similar conditions. However, when pH of the nornicotine solution was increased to 9.5, the rate of permeation was greatly increased, and under these conditions it compared favorably with the rate of permeation of nicotine from a solution containing the same proportion of free base. In this connection it is interesting to note that the L.D. 50 for nicotine on intravenous injection into rabbits is approximately 6 mg. per kg., while for nornicotine it is about 3 mg. However, on intraperitoneal injection, doses of 15 mg. per kg. of nicotine killed six of ten rabbits; whereas, nornicotine in molecular equivalent doses (13.7 mg. per kg.) killed only one of ten.* Thus, nornicotine is about twice as toxic as nicotine on intravenous injection; but when passage through additional membranes is necessary before its toxic properties can be exhibited, as on intraperitoneal injection, nornicotine is much less toxic than nicotine.

It has been well established by many experiments reported by various authors that the rates of absorption of other alkaloids are dependent on pH of the media containing the alkaloids. This suggests the interesting possibility that pharmacologic activities of many alkaloids may be increased or decreased between wide limits by relatively slight changes in molecular structure so that pK is increased or decreased as interest may dictate. Or, acidic drugs such as salicylic acid and neoarsphenamine, which do not permeate membranes readily, may be made to permeate by conversion into esters or derivatives which do not form ionizable salts at body pH.

SUMMARY

1. The collodion membrane containing lecithin and cephalin is permeable to molecules, but not to ions.

2. Any factor, such as changes in pH, esterification, and salt formation, which tends to change the degree of ionization, likewise changes the rate of permeation of such membranes.

3. Those substances which penetrate the brain when injected intravenously also permeate these artificial membranes; and those which do not penetrate the brain do not permeate such membranes.

4. In view of this close parallelism it is suggested that cell membranes containing phospholipids separating blood stream and brain substance constitute the so-called blood-brain barrier.

REFERENCES

Weatherby, J. H.: Concerning the Mechanism of Membrane Semipermeability, J. Cell. & Comp. Physiol. **21**: 1, 1943.

Friedemann, Ulrich: Blood-Brain Barrier, Physiol. Rev. **22**: 125, 1942.

Friedemann, Ulrich, and Elkeles, A.: The Blood-Brain Barrier in Infectious Diseases, The Lancet **227**: 775, 1934.

Friedemann, Ulrich: Further Investigations on the Blood-Brain Barrier, J. Immunol. 32: 97, 1937.

*Personal communication from Doctor P. S. Larson, Department of Pharmacology, Medical College of Virginia.

STUDIES ON THE DETOXICATION OF ORGANIC ARSENICAL COMPOUNDS

IV. The Protective Action of P-Aminobenzoic Acid Against Lethal Doses of Neoarsphenamine Without Inhibition of Trypanocidal Potency*

J. H. Sandground, Sc.D., and C. R. Hamilton, B.S.
Indianapolis, Ind.

INTRODUCTION

FROM extensive investigations in the field of bacterial metabolism, Woods[1] offered as a stimulating hypothesis the suggestion that it was by virtue of the chemical similarity obtaining between the two compounds that p-aminobenzoic acid acts as an inhibitor of the bacteriostatic property of sulfanilamide.

The structural relationship between certain phenylarsonate derivatives, such as atoxyl, tryparsamide, carbarsone, acetarsone, etc., on the one hand and p-aminobenzoic acid (hereafter abbreviated paba) on the other, is essentially comparable to that existing between paba and many of the sulfonamide compounds. In view of this analogy, we were impressed with the possibility that the trypanocidal potentials of the afore-mentioned arsenicals might be based on a mechanism similar in principle to the bacteriostatic mechanism postulated for the sulfonamides. To investigate this possibility an experiment was set up in which a group of rats infected with *Trypanosoma equiperdum* were treated with a massive (and usually lethal) dose of carbarsone to which a substantial quantity of paba had been added. Had there been an effective diminution of the trypanocidal action of carbarsone, these rats would have died in a few days from acute trypanosomiasis. Again, on the basis of our experience, we expected that they might succumb by about the fourth day from the toxic effect of an overdose of carbarsone. Actually the majority of the animals involved in this experiment developed few and only minor stigmas of arsenical poisoning. Furthermore they successfully overcame the hazard of death from trypanosomiasis.[2]

More interesting to us at the time than the failure of paba to inhibit the trypanocidal action of carbarsone was the entirely unsuspected possession by paba of a capacity to neutralize the lethal property of massive doses of carbarsone. This deduction was amply confirmed by further work not only with carbarsone, but also with such analogous pentavalent arsenicals as phenylarsonic acid, arsanilic acid, acetarsone, etc.[3] Later it was found that paba also has the capacity to protect rats against the lethal action of pentavalent phenyl stibonates, as exemplified by the German proprietory drug, stibosan.[4]

With a view to elucidating the mechanism underlying detoxication by paba, a large series of experiments were carried out. It was found[5] that a single administration of paba at the rate of 500 mg. per kilogram body weight can

*From the Lilly Research Laboratories.
Received for publication, July 8, 1943.

protect the lives of rats against several pentavalent arsenicals in quantities which considerably exceed their so-called "universal lethal dose" or $L.D._{100}$, e.g., 1500 mg. per kilogram of carbarsone, 450 mg. per kilogram of arsanilic acid, etc.

Of special interest and possibly of theoretical significance in connection with the mechanism of the detoxicating action was the finding that, whereas maximum beneficial effect is achieved when paba is injected simultaneously with, or even some time *prior to* the arsenical, when paba is introduced *subsequent to* the arsenical, its value is reduced almost in direct proportion to the interval that elapses.[6]

Unfettered by any theory as to the biochemical mechanism of the detoxicating process, our attention was directed early in the course of these investigations to determining broadly the potentialities and limitations of paba in conjunction with an array of other arsenical and antimonial compounds. Numerous experiments have been carried out with inorganic and organic arsenicals as represented by sodium arsenite, sodium cacodylate, mapharsen, arsphenamine, and neoarsphenamine, and the organic trivalent antimonial, tartar emetic. While paba was always used in substantial quantities as a detoxicant with the above-mentioned toxic substances, only in conjunction with neoarsphenamine were we able to obtain what seems to be unequivocal evidence of its capacity to interfere substantially with the lethal effect of large doses of the drug.

In view of the importance of neoarsphenamine in the treatment of syphilis, it is hoped that the publication of our experimental findings in this journal may be of interest.

EXPERIMENTAL PROCEDURE

Technically much greater difficulty is encountered in demonstrating clear-cut detoxication of neoarsphenamine than is to be experienced in proving detoxication against such drugs as carbarsone. This difficulty arises from the fact that, in contrast with the chemical purity of the pentavalent arsenicals and the crystalloidal nature of their aqueous solutions, the trivalent arsenobenzene compounds, such as arsphenamine and neoarsphenamine, are physicochemical complexes which are difficult to prepare in the state of purity indicated by their theoretical formulas. The toxicity rating and therapeutic efficacy of different lots of these compounds can only be established by biological assays. Because of the variation inherent in individual animals, this procedure, besides being expensive in terms of time, involves large numbers of animals, standardized so far as feasible by breeding, sex, age, and weight. The results of assays usually require the application of statistical methods for their correct interpretation.[7] However, even when tests with a homogeneous population of rats are performed with exacting attention to detail (e.g., in special air-conditioned rooms), nebulous and uncontrollable meteorologic factors seem to influence the mortality rates.[8] At best, the results obtained from such toxicity assays are to be interpreted as relative (to a standard) and can validly be presented only in terms of the median lethal dose ($L.D._{50}$).[9, 10]

The demonstration of the possession by any substance of a detoxicating action against neoarsphenamine must necessarily involve the same principles of

procedure, unless the detoxicating action is of such a high order that notwithstanding adverse conditions attending the assays, the results of protection tests are so obvious that statistical "booby traps" do not represent a hazard to their interpretation.

Largely as a consequence of prevailing conditions of supply as well as of other fortuitous circumstances, the experiments the results of which are recorded below were conducted with rats of a very heterogeneous assortment obtained through several animal dealers. Three lots of neoarsphenamine were used, each of a different manufacturer's brand purchased on the open market. On testing, one of these lots, "C," turned out to have a much higher toxicity for rats than did the other two. After preliminary toxicity tests with the particular lot of arsenical, protection experiments involving from 5 to 20 rats with an approximately equal number of unprotected controls were usually set up on one or two dosage rates. The assays were often repeated at the same dose rate or, if need be, with higher or lower doses of neoarsphenamine.

Despite the several handicaps to uniformity of results, it has been our experience that the outcome of a protection experiment was not essentially different, whether the assay was performed with the conventional young male 100-gram rats, of highly inbred strain and starved the night before testing, or whether we utilized the more readily available "mongrel" rats weighing up to 175 grams. The only precaution we have observed was to see that the drug was administered in quantities proportional to the rat's weight and to match rats of various weights equally between "treated" and "control" groups.

Obviously in order to secure meaningful data with a limited number of rats, it is necessary that the outcome of a protection experiment should show a significantly higher mortality rate in the control series than in the protected group. This calls for a rather nicely adjusted neoarsphenamine dosage approaching the maximum tolerance level. To go appreciably beyond this point, as we have on several occasions learned to our consternation, may lead to the death of all or nearly all of the animals, both "protected" and "controls." Another difficulty that can arise is characterized by the death of a large proportion of rats within a very few hours or even minutes of injection. This may happen irrespective of the dosage of arsenical and any protective treatment that may have been attempted. Agonal symptoms in such cases are referable to an acute vascular disturbance. Deaths of this order are clearly distinguishable from the normal, truly toxicologic fatalities that develop in from one to three or more days following injection. Wright et al.[11] found that the colloidal (in contradiction to crystalloidal) fraction of solutions of neoarsphenamine was responsible for the so-called immediate toxic reactions in 85 per cent of their rats that suffered precipitate deaths from respiratory failure. In some respects deaths of this type (which are, apparently, beyond the protective control of paba) resemble the "nitritoid crisis" or acute vasoparetic reaction, an ever present element of risk in the arsenic treatment of syphilis. Rather late in the course of our investigations we found that the incidence of deaths of the immediate type with the resultant waste of material and effort could be considerably reduced by employing subcutaneous injection in lieu of the usual intravenous (caudal or saphenous vein) route of introducing the drug.

The freshly prepared aqueous solution in 2 to 5 per cent concentration is

injected either simultaneously with, or not more than fifteen minutes after, the administration of a fresh 10 per cent aqueous solution of the sodium salt of p-aminobenzoic acid. Our rats have been accommodated in large communal cages, provided with ordinary commercial "chow," and kept under observation for at least ten full days before termination of the test.

TABLE I

COLLATED RESULTS OF EXPERIMENTS

PROTECTIVE ACTION OF p-AMINOBENZOIC ACID ON RATS RECEIVING MASSIVE DOSES OF NEOARSPHENAMINE

NEOARSPHENAMINE BRAND	NEOARSPHENAMINE DOSE MG./KG.	DOSE OF PABA MG./KG.	PABA-TREATED RATS NO. USED	PABA-TREATED RATS NO. SURVIVED	NONPROTECTED CONTROLS NO. USED	NONPROTECTED CONTROLS NO. SURVIVED
"A"	250 intravenously	500 intraperitoneally	11	6	9	3
	260 intravenously	2500 orally	22	14	15	6
	275 intravenously	1200 intraperitoneally	28	25	10	3
	275 intravenously	2000 orally	17	13	20	4
"B"	240 intravenously	1000 intraperitoneally	10	9	10	6
	250 intravenously	1000 intraperitoneally	9	9	10	3
	260 intravenously	1000 intraperitoneally	6	5	6	3
	280 intravenously	1000 orally	7	5	7	2
"C"	175 subcutaneously	1000 orally	10	4	21	1
		1000 intraperitoneally	50	37		
	180 subcutaneously	1000 intraperitoneally	40	31	27	1
	215 subcutaneously	1000 intraperitoneally	20	12	20	0

DISCUSSION

That paba possesses in substantial measure the power of protecting rats against the lethal effect of high doses of neoarsphenamine is, we believe, conclusively demonstrated from the results of experiments tabulated above, even without resorting to mathematical treatment of the figures to fortify this inference. Had we summarily eliminated from consideration those animals that died shortly after injection (as is sometimes done[10] in official assays of neoarsphenamine toxicity), the resulting figures would have supported the contention with even greater force. While "death" as the end point of a biologic reaction is by its very finality the most rigid measure that can be employed for the demonstration of protection (or the absence of protection) against a potent poison, the "all or none" nature of the criteria used in expressing the results of an experiment does not permit the introduction of fractional values into the situation. Such "fractional values" as are brought out by comparing the survival time of all rats in treated and control groups, or by contrasting their general condition and toxic reactions, are by no means insignificant. This is well illustrated by the accompanying photograph of three rats taken on the final day of the experiment, the tenth day after treatment with 175 mg. per kg. neoarsphenamine "Brand C." The normal-looking animals, 1 and 3, have received paba and are typical representatives of the protected survivors. Rat 2 is the only animal living out of 10 controls. It has lost weight (from 100 grams to 58 grams), and for several days appeared moribund. The fact that this animal escaped death, even though by a narrow margin, leads to its survival being credited in the protocol of results with the same unit value as any one of the paba-treated rats, e.g., 1 and 3, which have grown from 100 grams to 138 and

141 grams respectively without ever having been greatly indisposed by their arsenical medication. It is interesting to note in this connection that in our series of experiments the protective capacity of paba received its most rigorous test and appears to be most unequivocally demonstrated when a batch of neoarsphenamine (Brand C) of higher than usual toxicity was employed.

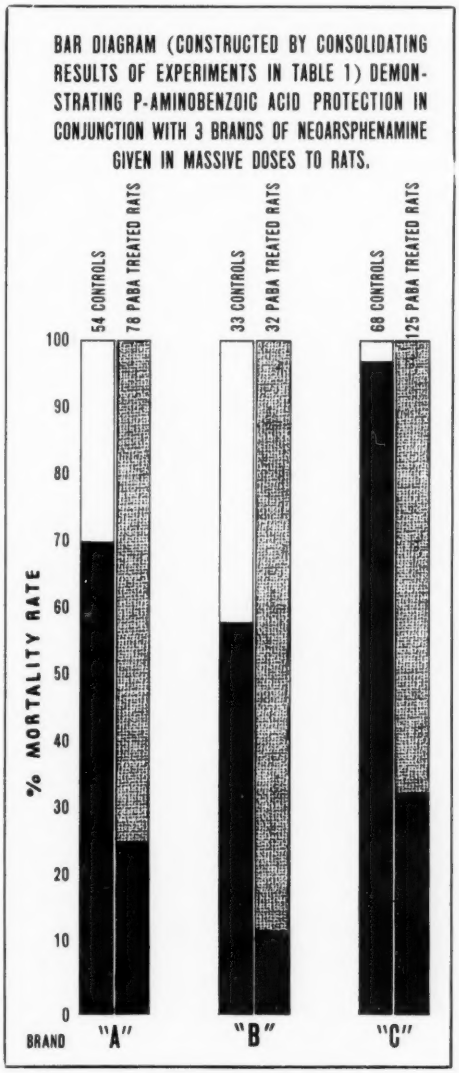

Fig. 1.

The clinical implication of the discovery that in rats paba has definite detoxicating value in conjunction with neoarsphenamine is difficult to evaluate without a critical trial. The subject is of sufficient importance to warrant a more extended consideration of its theoretical aspects than present space will permit. If paba is found to exercise a protective influence in human medication with neoarsphenamine, the question of its possible interference with the latter's treponemicidal activity will determine its clinical usefulness. In this connection,

the fact that in numerous experiments performed by us with *Trypanosoma equiperdum* paba in relatively large quantities was not found to diminish the parasiticidal value of neoarsphenamine may be of inferential significance.* This is to be contrasted with the experience of Durel,[13] who found that while solution of novarsenobenzol (=neoarsphenamine) in ascorbic acid appeared to reduce the toxicity of the arsenical in mice, the death rate from *Trypanosoma brucei* following treatment with a uniform dose of novarsenobillon was directly proportional to the amount of ascorbic acid contained in the mixture.† Loss of therapeutic efficacy with high doses of vitamin C in conjunction with arsenotherapy has also been suggested in clinical observations by Cormia.[14]

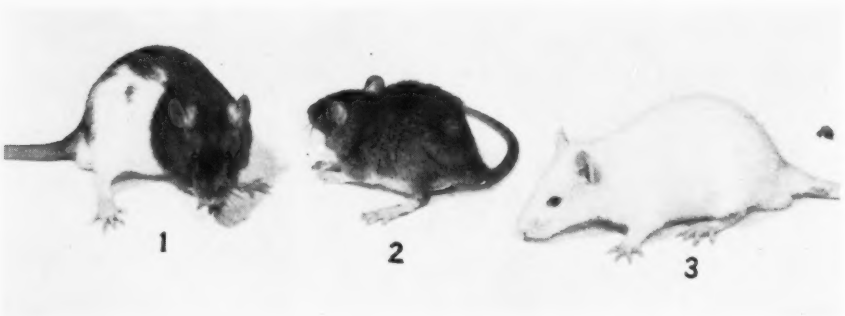

Fig. 2.—Photograph of rats treated with 175 mg. per kg. neoarsphenamine. Rat 2 is the sole survivor of 8 controls (see text). Rats 1 and 3 are typical of the 16 survivors out of 20 animals that received a simultaneous injection of *p*-aminobenzoic acid.

It should not be necessary to remark that a great chasm separates experience acquired with animals in the experimental laboratory from that which may be experienced in the clinic. Differences in the results obtained sometimes reflect fundamental differences in the metabolic processes of the animal species as reflected in their methods of disposing of certain chemical substances, e.g., benzoic acid. Frequently, however, the differences may be ascribed to the lack of parallelism between the conditions governing the laboratory experiment and those which are feasible when dealing with human lives. In advance it may be well to remark that in rat experiments the evidence of "paba" detoxication is best brought out by using doses of neoarsphenamine of a magnitude that has no parallel in the clinic unless it be in conjunction with the intensive arsenotherapy of syphilis. No evidence has been disclosed that would warrant the assumption that paba can act as an effective antidote against a toxic condition induced by the *prior* administration of arsenicals or that the concomitant administration of paba will eliminate the danger of the "nitritoid crisis" or counteract an "acquired sensitiveness" to arsenicals.

SUMMARY

Proceeding from a knowledge of the capacity of p-aminobenzoic acid to protect rats against the "certain lethal dose" ($L.D._{100+}$) of various pentavalent

*This inference is strengthened by the incidental observation of Osgood[12] who succinctly remarks "p-aminobenzoic acid does not interfere with the action of neoarsphenamine as it does with the action of the drugs of the sulfanilamide group."

†Incidentally, it may be recorded that in several parallel experiments we have not found ascorbic acid to have detoxicating power comparable with that of paba.

arsenical and antimonial compounds, a large series of experiments have been performed to determine the scope of paba protection in conjunction with several trivalent arsenical and antimonial drugs.

It appears that paba has no detoxicating capacity against inorganic arsenous acid; the arsenoxide mapharsen; the arsenobenzene arsphenamine; or the trivalent organic antimonial tartar emetic.

Against neoarsphenamine, protection experiments with a large series of unselected rats are reported in this paper. The results show a very substantial reduction in the mortality rate without a concomitant inhibition of its parasiticidal potency as measured by its effect on trypanosomiasis in the rat.

The clinical implication of these findings, especially in connection with the intensive treatment of syphilis, warrant further investigation.

REFERENCES

1. Woods, D. D.: The Relation of p-Aminobenzoic Acid to the Mechanism of the Action of Sulphanilamide, Brit. J. Exper. Path. **21**: 74, 1940.
2. Sandground, J. H.: p-Aminobenzoic Acid Detoxication of Carbarsone (p-Carbamino Phenyl Arsonic Acid) and Certain Other Pentavalent Phenyl Arsonates Administered in Massive Doses to Rats, Science **97**: 73, 1943.
3. Sandground, J. H., and Hamilton, C. R.: Studies on the Detoxication of Organic Arsenical Compounds. I. Detoxication by Means of p-Aminobenzoic Acid of Certain Pentavalent Arsenical Drugs Given in Massive Doses to Rats, J. Pharmacol. & Exper. Therap. **78**: 109, 1943.
4. Sandground, J. H.: Detoxication of a Substituted Phenyl Stibonate in the Rat by Administration of p-Aminobenzoic Acid, Proc. Soc. Exper. Biol. & Med. **52**: 188, 1943.
5. Sandground, J. H., and Hamilton, C. R.: Studies on the Detoxication of Organic Arsenical Compounds. II. Correlation of the Quantity of p-Aminobenzoic Acid Required to Protect Rats Against High Doses of Carbarsone and Arsanilic Acid, J. Pharmacol. & Exper. Therap. **78**: 203, 1943.
6. Sandground, J. H.: Studies on the Detoxication of Organic Arsenical Compounds. III. The Time Factor Influencing p-Aminobenzoate Protection of Rats Receiving Lethal Doses of Phenyl Arsonates, J. Pharmacol. & Exper. Therap. **78**: 209, 1943.
7. Morrell, C. A., Chapman, C. W., and Allmark, M. G.: A Study of the Effect of Age, Weight, Sex, and Dose on Length of Survival of Albino Rats Infected With *Trypanosoma equiperdum* With Particular Reference to Variations in Host, Am. J. Hyg. **25**: 232, 1937.
8. Nedsel, J. A.: Daily Variations in Toxicity of Neoarsphenamine in White Rats, J. Lab. & Clin. Med. **27**: 720, 1942.
9. Trevan, J. W.: The Error of Determination of Toxicity, Proc. Roy. Soc., London, ser. B. **101**: 483, 1927.
10. Hawking, F.: The Standardization of the Therapeutic Potency of Neoarsphenamine and Sulpharsphenamine, Quart. J. Pharm. & Pharmacol. **16**: 13, 1943.
11. Wright, H. N., Biedermann, A., Hanssen, E., and Cooper, C. I.: Physico-Chemical Properties of the Arsphenamines in Relation to Toxicity and Therapeutic Efficiency, J. Pharmacol. & Exper. Therap. **73**: 12, 1941.
12. Osgood, E. E.: Noarsphenamine Therapy of Bacterial Infections, Arch. Int. Med. **69**: 746, 1942.
13. Durel, M. P.: Influence de la vitamin C sur la toxicité et l' activité du novarsenobenzol chez la souris, Bull. Soc. franç. de dermat. et syph. **44**: 1077, 1937.
14. Cormia, F. F.: Postarsphenamine Dermatitis: The Relation of Vitamin C to Production of Arsphenamine Sensitiveness and Its Use as an Adjunct to Further Arsphenamine Therapy, J. Invest. Dermat. **4**: 81, 1941.

CLINICAL CHEMISTRY

ABSORPTION, EXCRETION, AND DISTRIBUTION OF SULFAMETHAZINE (2-SULFANILAMIDO-4-6-DIMETHYL-PYRIMIDINE) IN MAN*

JOHN K. CLARK, M.D., FRANKLIN D. MURPHY, M.D., AND HARRISON F. FLIPPIN, M.D., PHILADELPHIA, PA.

THERE has been a marked improvement in the therapeutic efficacy and lack of toxicity in the series of sulfonamides that has been introduced for clinical use since sulfanilamide. Among the more recent of these compounds, sulfamerazine (2-sulfanilamido-4-methyl-pyrimidine) and sulfamethazine (2- sulfanilamido-4-6-dimethyl-pyrimidine) have warranted clinical trial because of their therapeutic effectiveness against various experimental infections and the relatively high solubility of their acetylated forms.

Since the intelligent use of these drugs requires an understanding of their behavior when administered to man, we have investigated their fate in a group of patients. In a recent paper,[1] we reviewed the experimental data pertaining to sulfamerazine and presented observations dealing with its behavior in man; this suggested that sulfamerazine is pharmacologically satisfactory. At this time we are reporting the results of a similar study on sulfamethazine† and have made a comparison of the two drugs on the basis of their absorption, excretion, and distribution in human beings.

Sulfamethazine (2-sulfanilamido, 4-6-dimethyl-pyrimidine), described by several groups of investigators,[2-4] is a dimethyl homologue of sulfadiazine. The therapeutic effectiveness of sulfamethazine against experimental infection has been investigated by Rose, Martin, and Bevan[4] and found to be highly active against pneumococcal and streptococcal infections in mice. Pharmacologic studies, both in mice and human beings,[4] indicate that the drug is freely and rapidly absorbed from the gastrointestinal tract and it is stated to be slowly eliminated from the blood stream. Furthermore, it was suggested that, because of its high solubility, sulfamethazine is unlikely to cause renal damage.[4] Preliminary clinical trials with the compound have shown that it is highly effective in pneumococcal, meningococcal, and gonococcal infections and is less toxic than is sulfapyridine.[5, 6]

The methods used in this investigation were the same as those employed in the sulfamerazine study.[1]

*From the Department of Medicine and the William Pepper Laboratory of the Hospital of the University of Pennsylvania.

This work was aided by a grant from the Sharp and Dohme Laboratories, Glenolden, Pa.
Received for publication, July 13, 1943.

†The sulfamethazine used in this study was supplied us by Sharp and Dohme Laboratories, Glenolden, Pa.

RESULTS

A. Absorption.—

1. *Single Oral Dose.*—Nine patients (Nos. 1 to 9, Table I) were given a single 3 Gm. dose of the drug. At the end of 2 hours the average concentration of free sulfamethazine in the serum was 11.1 mg. per 100 c.c., and, after 4 hours, the average concentration reached a peak of 12.0 mg. per 100 c.c. Thereafter, the level began to fall, and at the end of 12 and 24 hours it averaged 6.1 and 2.2 mg. per 100 c.c. respectively. Acetylation of the drug varied. At the end of 6 hours an average of 21.6 per cent of the total drug was in the acetylated form; at 12 hours, 37.1 per cent, and after 24 hours, 48.8 per cent.

2. *Single Intravenous Dose.*—Two patients (Nos. 10 and 11, Table I) were each given 3 Gm. of sulfamethazine sodium in 5 per cent solution in sterile distilled water intravenously. Within one-half hour the average level of free drug was 15.3 mg. per 100 c.c.; in 2 hours the concentration averaged 11.4 mg.; after 4 hours, 9.5 mg.; and, at the end of 24 hours, 1.9 mg. per 100 c.c.

3. *Single Subcutaneous Dose.*—Two patients (Nos. 12 and 13, Table I) were each given 3 Gm. of sulfamethazine sodium, 0.5 per cent concentration in 0.85 per cent sodium chloride solution, subcutaneously. The average concentration of free drug reached a peak of 8.5 mg. per 100 c.c. at 2 hours, within 4 hours the level was 7.1 mg., after 6 hours, 5.9 mg., and at the end of 24 hours, 0.5 mg. per 100 c.c. Acetylation of the drug after parenteral administration very closely paralleled that found with oral administration.

4. *Multiple Dosage.*—Twelve patients, suffering from several diseases, including pneumococcal pneumonia, meningococcal septicemia, puerperal sepsis, postoperative pneumonia, were treated with sulfamethazine for varying periods of time and with different dosage schedules. In general, they were given 2 to 4 Gm. by mouth, followed by 1 Gm. every 4 to 6 hours. Serum levels varied widely from 1.0 to 23.0 mg. per 100 c.c. of the free drug with an average acetylation of 38.3 per cent (range 0.8 to 83.5 per cent). While serum concentrations of sulfamethazine varied greatly from person to person, they were constant for the same individual.

B. Excretion.—

Urinary excretion of the drug was measured (Table I). After the oral administration of a single 3 Gm. dose, from 17.1 to 55 per cent (average 40.1 per cent) was recovered in the first 12 hours; by the end of 24 hours from 40.8 to 90 per cent (average 71.8 per cent) had been excreted, and, at the end of 48 hours from 67.6 to 98.8 per cent (average 86.2 per cent) of the ingested drug was recovered from the urine. During the first 24 hours an average of 70.9 per cent of the excreted drug was in the acetylated form, and, after 48 hours, 77.3 per cent was acetylated drug. Similar results were obtained when the drug was given parenterally (Table I).

C. Distribution.—

1. *Cerebrospinal Fluid.*—Four patients (Nos. 14, 15, 16, and 17, Table II) were given the drug by mouth, and cerebrospinal fluid and blood specimens were obtained at varying intervals. Six hours after the administration of 3 Gm. of sulfamethazine, the ratio of the cerebrospinal fluid level of free drug to that

TABLE I

FATE OF SULFAMETHAZINE ADMINISTERED BY VARIOUS ROUTES

ABSORPTION AND EXCRETION OF SINGLE ORAL DOSE (3 GM.) OF SULFAMETHAZINE

NO.	PATIENT AGE (YEARS) WT. (LBS.)	TIME (HRS.)	SERUM SULFAMETHAZINE FREE (MG. PER 100 C.C.)	SERUM SULFAMETHAZINE TOTAL (MG. PER 100 C.C.)	SERUM SULFAMETHAZINE ACETYLATED (%)	URINE SULFAMETHAZINE FREE (MG. PER 100 C.C.)	URINE SULFAMETHAZINE TOTAL (MG. PER 100 C.C.)	URINE SULFAMETHAZINE ACETYLATED (%)	URINE CUMULATIVE EXCRETION (%) FREE	URINE CUMULATIVE EXCRETION (%) TOTAL	URINE VOLUME (C.C.)
1	R. P.	2	9.0	9.2	2.2						
	36	4	11.2	12.0	6.7						
	160	6	11.3	12.9	12.4						
		12	6.5	9.0	27.8	83.0	130.0	36.2	20.7	32.5	750
		24	2.4	3.5	31.4	74.3	145.0	48.8	35.6	54.0	600
		48				17.5	52.5	66.7	42.2	80.4	1135
2	J. S.	2	15.8	15.8	0						
	43	4	14.8	16.1	8.1						
	130	6	14.0	15.7	10.8						
		12	9.5	11.4	16.7	144.4	244.5	41.1	21.2	35.8	440
		24	3.6	4.9	26.1	117.0	239.5	51.2	36.4	67.1	390
		48				17.6	67.3	73.8	39.7	79.2	540
3	A. P.	2	2.5	2.5	0						
	17	4	7.5	7.5	0						
	120	6	12.6	13.1	3.9						
		12	11.4	12.0	5.0	28.6	45.0	36.5	10.9	17.1	1140
		24	6.0	8.2	26.8	73.0	148.5	50.8	22.2	40.8	480
		48				23.8	66.2	64.1	30.5	67.6	1040
4	K.	2	17.0	18.3	7.2						
		4	15.0	15.7	4.5						
		6	14.1	15.0	6.0						
		12	8.0	10.0	20.0	30.3	53.0	42.8	19.2	33.6	1900
		24	3.8	3.5		30.8	90.0	65.8	33.3	74.8	1375
		48				10.1	40.0	74.8	39.3	98.9	1800
5	O.	2	8.5	10.6	19.8						
		4	11.9	16.0	25.7						
		6	11.7	17.7	33.9						
		12	5.3	12.8	58.7	44.5	170.5	73.9	10.4	40.9	720
		24	1.5	4.7	68.1	12.0	70.0	82.9	15.6	71.5	1310
		48				4.3	25.0	82.8	18.7	89.9	2200
6	M.	2	11.5	15.4	25.3						
	35	4	13.8	18.7	26.2						
	140	6	10.5	17.5	40.4						
		12	3.1	9.0	65.6	128.3	530.0	75.8	13.3	54.7	310
		24	1.0	3.8	73.7	70.3	540.0	86.8	18.0	90.7	200
		48				15.0	98.0	84.7	19.1	97.6	210
7	K. C.	2	12.7	18.0	29.4						

7	K. C.	2	12.7	18.0	29.4						
	20	4	9.8	12.1	19.0						
	140	6	4.5	8.9	49.4						
		12	.5	4.5	88.9	60.0	445.0	86.5	6.4	47.5	320
		24	.3	2.0	85.0	5	135.0	96.3	7.8	84.4	820
		48				1.3	9.3	86.0	15.5	89.9	1775
8	F. W.	2	16.2	17.2	5.8						
	35	4	12.9	14.0	7.9						
	160	6	12.5	13.9	10.1						
		12	6.5	8.5	23.5	42.5	90.5	53.6	20.9	43.4	1440
		24	1.5	4.5	66.7	59.0	159.5	63.0	29.9	70.0	500
		48				12.1	43.1	71.9	43.8	75.0	345
9	L. S.	2	6.3	7.3	13.7						
	30	4	11.0	14.6	24.6						
	150	6	9.6	13.9	30.9						
		12	3.8	10.3	63.1	63.0	412.5	84.6	8.4	55.0	400
		24	0.1	3.5	97.1	42.5	455.0	90.7	13.0	90.6	235
		48				4.0	41.3	90.3	13.6	97.2	475
					INTRAVENOUS INJECTION OF SINGLE 3 GM. DOSE OF SULFAMETHAZINE SODIUM							
10	R. B.	½	13.0	14.5	10.4						
	35	2	7.9	11.4	30.7						
	175	4	5.5	8.3	34.9						
		6	4.5	6.9	34.8						
		12	.8	2.8	71.4	27.5	107.5	74.4		41.2	1150
		24	.3	1.4	21.4	12.5	108.8	88.4		61.1	550
		48				3.2	18.4	83.1		71	1550
11	S. G.	½	17.6	17.8	1.1						
	40	2	14.9	15.9	6.3						
	160	4	13.5	13.8	2.2						
		6	11.3	12.3	8.1						
		12	6.5	7.1	8.4	22.5	47.5	54.7		53.8	3250
		24	3.5	4.0	12.5	52.8	77.5	31.8		74.5	800
		48				14.0	25.7	45.5		75	1665
					SUBCUTANEOUS INFUSION OF SINGLE 3 GM. DOSE OF SULFAMETHAZINE SODIUM							
12	R. E.	2	9.2	9.3	1.2						
	19	4	9.3	9.8	5.1						
	130	6	8.3	8.6	3.5						
		12	4.9	6.4	23.4	21.8	46.0	52.6			1565
		24	0.7	3.5	80.0	29.5	106.0	72.2			850
		48				6.0	27.2	77.9		72	1900
13	S. H.	2	7.8	11.5	32.2						
	40	4	4.9	9.9	50.5						
	165	6	3.5	8.0	56.3						
		12	0.5	3.0	83.3	25.0	113.5	77.9			1365
		24	0.1	0.5	80.0	5.0	52.5	94.1			675
		48				0.6	6.0	90.0		67	1660

in the serum was 0.04 (No. 14). After 12 hours, following a similar dose of the drug, the ratio was 0.58 (No. 15). Two patients were given multiple doses of sulfamethazine, and after 24 hours (No. 16) and 11 days (No. 17) the ratios were 0.33 and 0.29 respectively.

2. *Pleural and Ascitic Fluid.*—In three patients (Nos. 18, 19, and 20, Table II) who received sulfamethazine pleural or ascitic fluid and blood samples were taken at varying intervals. Ratios of 0.86 and 0.53 were obtained in pleural fluid 12 hours (No. 18) and 4 days (No. 19) following 3 and 20 Gm. of the drug respectively. In one patient (No. 20), after 20 Gm. of the drug, the ratio of 0.51 in ascitic fluid was obtained.

3. *Erythrocytes.*—Simultaneous determinations of sulfamethazine concentration in serum and whole blood were performed in a single patient (Table III). The results in this instance show that the drug penetrates the red blood cells.

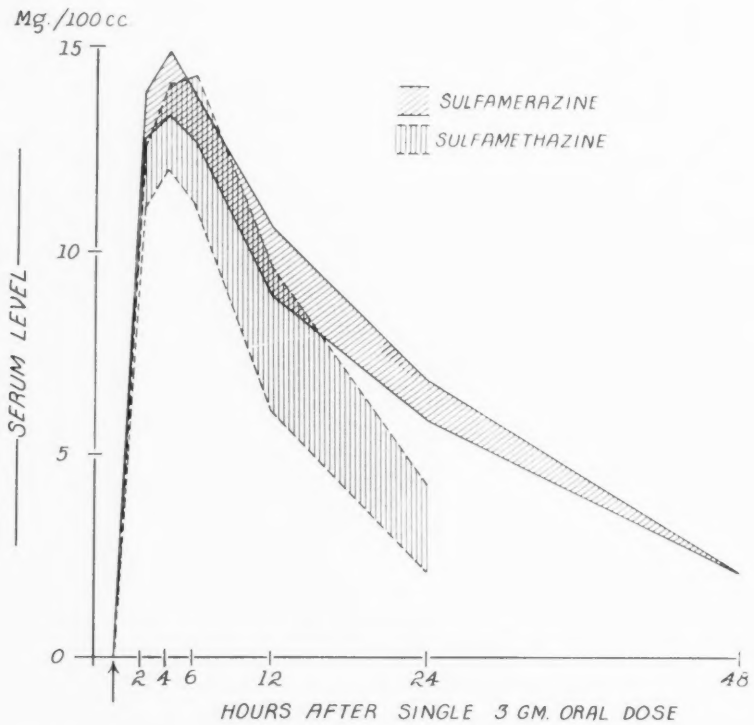

Fig. 1.—Comparison of average serum concentrations obtained in 9 subjects receiving 3 Gm. of sulfamethazine with those obtained in 8 subjects receiving a similar amount of sulfamerazine. In each curve the lower line indicates free drug and the upper line, total drug.

DISCUSSION

Comparison of these data on sulfamethazine with recent studies in this laboratory on sulfamerazine[1] indicates that the two drugs are absorbed from the gastrointestinal tract with equal rapidity (Fig. 1). The rate of excretion of both free and acetyl-sulfamethazine is greater than that found with sulfamerazine and its acetyl derivative (Fig. 2). The two drugs diffuse readily into various body tissues and tissue fluids. In view of the greater solubility of

TABLE II

FREE SULFAMETHAZINE CONCENTRATION IN VARIOUS BODY FLUIDS

BODY FLUID	NO.	PATIENT	HOURS AFTER INITIAL DOSE	NO. OF DOSES	TOTAL DOSE (GM.)	SERUM (MG. PER 100 C.C.)	BODY FLUID (MG. PER 100 C.C.)	CONCENTRATION RATIO (BODY FLUID/SERUM)
Cerebrospinal Fluid	14	R. G.	6	1	3	7.5	0.3	0.04
	15	R. S.	12	1	3	1.9	1.1	0.58
	16	M. B.	24	4	7	9.5	3.1	0.33
	17	M. L.	11 days	39	41	21.9	6.3	0.29
Pleural Fluid	18	W. B.	12	1	3	2.8	2.4	0.86
	19	B. S.	4 days	18	20	13.4	7.1	0.53
Ascitic Fluid	20	B. S.	4 days	18	20	13.4	6.8	0.51

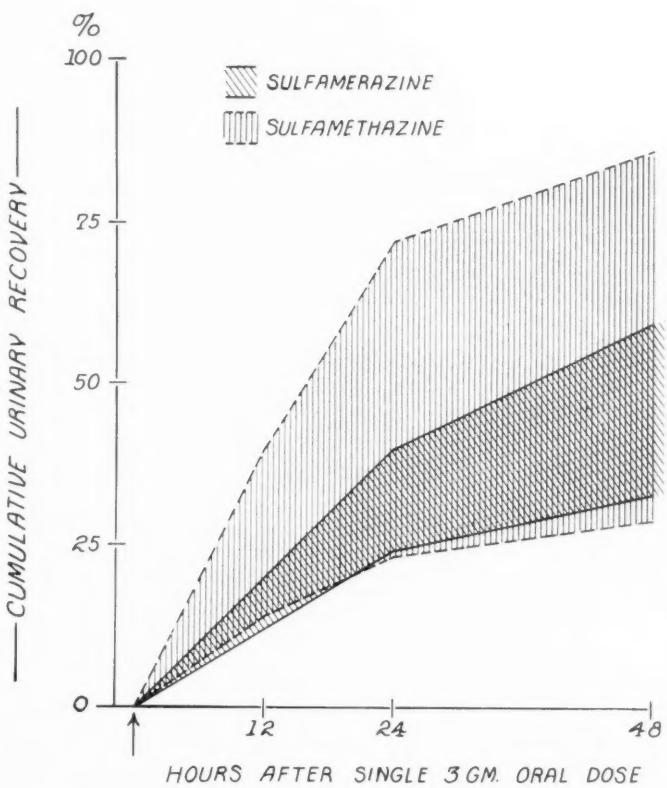

Fig. 2.—Comparison of average cumulative urinary excretions obtained in 9 subjects receiving 3 Gm. of sulfamethazine with those obtained in 8 subjects receiving a similar amount of sulfamerazine. In each curve the lower line indicates free drug and the upper line, total drug.

sulfamethazine,[3] the incidence of urinary complications may be less than with sulfamerazine. Because of its rapid excretion and high acetylation, both in serum and urine, sulfamethazine appears to be less satisfactory from a pharmacologic viewpoint than sulfamerazine. The above findings are in keeping with those recently reported by Welch and his associates.[8]

TABLE III

DISTRIBUTION OF SULFAMETHAZINE BETWEEN SERUM AND RED BLOOD CELLS
SULFAMETHAZINE CONCENTRATION IN MG. PER 100 C.C.

NO.	PATIENT	DAY OF DRUG	WHOLE BLOOD		SERUM		CELLS (CALCULATED)		PACKED CELL VOLUME (%)
			FREE	TOTAL	FREE	TOTAL	FREE	TOTAL	
21	M. L.	11	15.5	16.3	21.9	23.9	6.0	5.0	40

SUMMARY

Data are presented concerning the absorption, excretion, and distribution of sulfamethazine in humans. They show: (1) that sulfamethazine is readily absorbed from the gastrointestinal tract; (2) that it is rapidly excreted in the urine; and (3) it readily diffuses into pleural, ascitic, and cerebrospinal fluids and into the red blood cells. Comparison with the behavior of sulfamerazine shows that higher blood concentrations are sustained longer with sulfamerazine than after similar amounts of sulfamethazine.

REFERENCES

1. Murphy, F. D., Clark, J. K., and Flippin, H. F.: Studies on 2-Sulfanilamido-4-Methyl-Pyrimidine (Sulfamerazine, Sulfamethyldiazine) in Man. I. Absorption, Distribution, and Excretion, Am. J. M. Sc. **205**: 717, 1943.
2. Caldwell, W. T., Kornfeld, E. C., and Donnell, C. K.: Substituted 2-Sulfanilamido-pyrimidines, J. Am. Chem. Soc. **63**: 2189, 1941.
3. Roblin, R. O., Jr., Winnek, P. S., and English, J. P.: Studies in Chemotherapy; Sulfanilamidopyrimidines, J. Am. Chem. Soc. **64**: 567, 1942.
4. Rose, F. L., Martin, A. R., and Bevan, H. G. L.: Sulphamethazine 2-4-AminoBenezene-sulphonylamino-4:6-dimethylpyrimidine) A New Heterocyclic Derivative of Sulphanilamide, J. Pharmacol. & Exper. Therap. **77**: 127, 1943.
5. McCartney, D. W., Smith, G. S., Luxton, R. W., Ramsay, W. A., and Goldman, J.: Sulphamethazine: Clinical Trial of a New Sulphonamide, Lancet **1**: 639, 1942.
6. Jennings, P. A., and Patterson, W. D.: Sulphamethazine. Clinical Trials in Children, Lancet **2**: 308, 1942.
7. Clark, J. K., Flippin, H. F., and Murphy, F. D.: Studies on 2-Sulfanilamido-4-Methyl-Pyrimidine (Sulfamerazine, Sulfamethyldiazine) in Man. II. Toxic Manifestations, Am. J. M. Sc. **205**: 846, 1943.
8. Welch, A. D., Mattis, P. A., Latven, A. R., Benson, W. M., and Shiels, E. H.: Sulfamerizine (2-Sulfanilamido-4-methylpyrimidine). I. A Comparison of Sulfamerizine with Sulfadiazine on the Basis of Absorption, Excretion, and Toxicity, J. Pharmacol. & Exper. Therap. **77**: 357, 1943.

STUDIES IN THE PHARMACOLOGY OF MERCURY

III. Histochemical Demonstration and Differentiation of Metallic Mercury, Mercurous Mercury, and Mercuric Mercury*

W. C. Hand, B.S., B. B. Edwards, B.S., and E. R. Caley, Ph.D.†
New Brunswick, N. J.

MERCURY and its compounds have been used for many years in the prevention and treatment of syphilis; yet a review of the literature discloses little material on methods for observing the valency changes which may take place during and after the absorption of mercury and its derivatives from the skin and other depots of administration. The development and utilization of differential methods for demonstrating mercury in the organs of the body should serve to extend our knowledge of the pharmacology of this element and perhaps lead ultimately to more efficacious clinical preparations. In this paper we present some new histochemical methods together with a review and extension of certain older ones.

CHEMICAL CONSIDERATIONS

General.—It is self-evident that a histochemical reagent should react in a characteristic manner with the substance to be detected. As Christeller[1] points out, this is easy in the case of iron, since there are a number of brilliant-hued specific chemical reactions. In spot tests[2] mercury likewise gives fairly specific color reactions with such reagents as diphenylcarbazide and dithizone. Our experiments with the above reagents on sections known to contain mercury compounds were disappointing. The color was either too faint or too evanescent. Precipitation with sulfides according to the procedures of Justus[3] and of Almkvist[4] also gave inconclusive results. The particle size of mercury sulfides formed in tissues may be extremely small, so that it is difficult to differentiate them from hemosiderin and other pigments. The use of iodine and iodide proved fruitless, as previously noted by Christeller because the entire section is stained. We ultimately confirmed the conclusion previously reached by Lombardo[5] and by Christeller that the reduction of mercury in the tissues to the metallic form is the simplest and most reliable histochemical procedure.

Metallic Mercury.—As has been pointed out by Zwick[6] and by Emich,[7] the microscopic appearance of finely divided mercury is highly characteristic. The particles are spherical, black, and nonrefractile. When sufficiently large, an image of the lens of the objective is reflected from their centers. Their very roundness makes them subject to ready displacement, however, and the section is best cut rather thick (15 microns) if free mercury is suspected, in order that a comparatively undisturbed inner zone may be available for inspection.

Zwick demonstrated that metallic mercury is present in the subcutaneous

*Part I of Studies in the Pharmacology of Mercury, J. Am. Pharm. A. (Scient. Ed.) 32: 255, 1943. Part II, in press.

†Contribution of Wallace Laboratories, Inc., New Brunswick, N. J.

Received for publication, August 5, 1943.

tissues after inunction of the element in a salve vehicle. We have confirmed this observation. He pointed out that the globules react with components of tissue fluids and lose their spherical shape. According to Christeller, they dissolve completely in tincture of iodine and this characteristic reaction may be observed under the microscope. We have observed further that solutions of chlorauric acid react with the minute spheres, causing at first a loss of gloss and a roughening of surface; this is followed by a gradual disintegration of the spheres as the gold amalgam forms. These changes were observed microscopically in sections of tissue. The above-mentioned characteristic microscopic appearance and microchemical behavior permit positive identification of the metal.

Mercurous Mercury.—Compounds containing mercurous mercury are usually colorless. Most mercurous salts, for example, the acetate, combine or mix with blood and other protein systems without the formation of opaque or colored bodies. For histochemical purposes, it is accordingly necessary to render the compounds visible. This can be done, even in the presence of mercuric mercury, by employing a reagent with a selective reducing action, so that only the mercurous mercury is reduced to the metal. Thioglycollic acid exhibits this differential selective reducing effect. In test tube experiments calomel is reduced immediately. Under the microscope, a suspension of calomel crystals is transformed almost instantaneously to a dispersion of metallic globules, when a drop of thioglycollic acid is introduced under the cover slip.

Mercuric salts are not so reduced. They react with thioglycollic acid to form compounds which appear as colorless to yellow crystals. We have not attempted to determine the precise nature of these compounds as they are found in histochemical reactions. It has been reported, however, by Claesson[8] that mercuric compounds react with thioglycollic acid to form either mercurithioglycollic acid ($Hg[S.CH_2COOH]_2$) or mercuric thioglycollate ($Hg[S.CH_2COO]_2Hg$), according to conditions. It is likely, that in the histochemical testing where an excess of reagent is normally present only the first of these two compounds is formed in appreciable amount. The presence of crystals of mercurithioglycollic acid does not interfere with the histochemical interpretation of the sections. Although the appearance of the crystals is rather characteristic, we do not recommend that they be regarded as an index of the mercuric mercury present. This reservation is based on the fact that they may dissolve and recrystallize at a point remote from the original site of the mercuric compound.

Tissue sections which contain mercurous mercury exhibit a prompt formation of mercury when a thioglycollic acid solution is introduced under the cover slip. The gradual formation of these globules can be followed visually. Should mercuric mercury also be present in appreciable amount, characteristic yellowish crystals begin to form after about five minutes.

Mercuric Mercury.—It was established by Lombardo and confirmed by Christeller that mercury is precipitated from tissues by stannous chloride solutions. This is a well-known chemical phenomenon which can be demonstrated readily in the test tube. Stannous chloride solutions reduce both mercurous and mercuric salts (including the thioglycollic acid mercury salts) to free mercury. It is thus possible, first, to examine a section for free mercury, then, to detect the mercurous mercury with thioglycollic acid, and, finally, to

detect the remaining mercuric mercury with stannous chloride. In actual practice, however, we prefer to treat three separate serial sections with the several reagents as described below. Sodium stannite solutions also are effective, but they macerate the tissue.

SUITABLE REAGENTS

General.—The reagents used in these studies were developed by a process of trial and error. It is not claimed, nor maintained, that they represent the best possible embodiment of the reducing agents. They have been tested, however, on thousands of sections from more than fifty animals including rabbits, guinea pigs and mice, with satisfactory results. In general, no counterstains have been employed. In our experience counterstains tend to displace the mercury globules.

Metallic Mercury.—As has been noted elsewhere, it is not necessary to use a reagent to demonstrate the presence of free mercury.[1] Inasmuch as this work has been done almost exclusively with frozen sections, however, a clearing agent is employed. Its formula is as follows:

Formula I:	Tartaric acid	5 Gm.
	Glycerin	100 milliliters
Preparation:	Add the acid to the glycerin and agitate. The crystals will dissolve overnight.	

The above solution removes hemosiderin deposits and provides sections which exhibit adequate cellular detail without counterstaining.

As a confirmatory test to dissolve the mercury, official tincture of iodine may be used.

Formula II:	Iodine	70 Gm.
	Potassium Iodide	50 Gm.
	Distilled Water	50 Gm.
	Ethyl Alcohol, 95 per cent	To make 1000 milliliters
Preparation:	Dissolve potassium iodide in the water, add the iodine and agitate until solution is effected. Then add sufficient alcohol to make 1000 milliliters and mix thoroughly.	

The gold amalgam reaction is sometimes useful in fresh frozen sections of the skin because it does not stain initially as intensely as does iodine. This test is occasionally useful when confusion is experienced with skin pigments.

Formula III:	Chlorauric acid	1 gram
	Distilled water	99 milliliters
Preparation:	Dissolve the chlorauric acid in the distilled water in the cold with stirring. This reagent should be stored in a dark bottle.	

Mercurous Mercury.—The following solution of thioglycollic acid has been found satisfactory:

Formula IV:	Thioglycollic acid	1 milliliter
	Glycerin	9 milliliters
Preparation:	The above are completely miscible.	

Mercuric Mercury.—Stannous chloride reagent of the following composition may be stabilized by adding a few grams of metallic tin to the final solution which should be kept in a well-stoppered bottle.

Formula V:	Stannous chloride	5 Gm.
	Tartaric acid	5 Gm.
	Glycerin	100 milliliters
Preparation:	Combine and heat until the solution is clear.	

HISTOLOGIC TECHNIQUE

Throughout this study, every attempt was made to avoid artefactual changes in the tissues. The extended fixation of tissue advocated by Christeller and Lombardo may result, as the former author noted, in a *leaching out* of mercury compounds. Also, the immersion of whole sections permits diffusion of mercury compounds within the specimen with the resultant introduction of unpredictable inaccuracies in the final readings. Furthermore, the usual histologic fixations may similarly contribute an unknown artefactual change in the specimens. As a matter of fact, Christeller observed that formalin and alcohol decreased his yield. In work with frozen sections of material previously fixed in alcohol or formalin, we noted, histochemically, that our reagents usually produced a precipitate on the exposed surface of the sections only. For these reasons and in the interests of more rapid work, unfixed frozen sections were employed in our experiments.

In our standard practice the sections are taken immediately after sacrificing the animals, because experience showed a loss of clarity in cellular detail if the specimens were left at room temperature more than thirty minutes after death. It is believed that the small interval between sacrifice of the animal and completion of the finished section contributed in no small measure to the reproducibility of the findings.

The procedure is the same with Reagents I, IV, and V. The fresh section is placed on the freezing microtome over a piece of damp tissue paper which aids in the adherence of the specimen. Sections of about 15 microns are then cut, placed on a clean slide by means of a brush, and left uncovered until dry. This is necessary in case of Reagent V, to avoid precipitating stannous hydroxide. When dry, one drop of the reagent is added, a cover slip is placed over it, and the excess of the reagent blotted away. Then the edges of the cover slip are sealed with commercial gold size.* It is imperative that the cover slips be well sealed because of the pronounced humectant and creeping properties of the glycerin. The entire sectioning, mounting, and testing with reagent can be finished within ten minutes. The reagents act promptly and the slides are ready for reading ten minutes after they are made. Slides prepared with Reagent I, which is routinely used as a control, as well as those prepared with Reagent V, usually remain unchanged for at least two weeks.

It may be noted here that tissue sections which contain both mercurous and mercuric ions can give a misleading impression if they are read too long after the application of the thioglycollic acid reagent (IV). The reason for this is that free mercury will react with mercuric mercury in a complex oxidation-reduction

*Commercial gold size is the type of adhesive used for applying gold foil to plate glass.

system. Additional free mercury is liberated during the process. This reaction is slow, in the sections, so that no visible change is observed over a period of several hours. Twenty-four hours later, however, the globules may have become more numerous and larger. One may take advantage of this reaction, if one wishes to establish the presence or absence of submicroscopic quantities of mercurous mercury in a tissue known to contain mercuric ions. If there has been any mercurous ion present it will have manifested itself as mercury after several days because of the reaction described above. Under ordinary circumstances, we recommend that slides treated with reagent IV be photographed or read within a few hours.

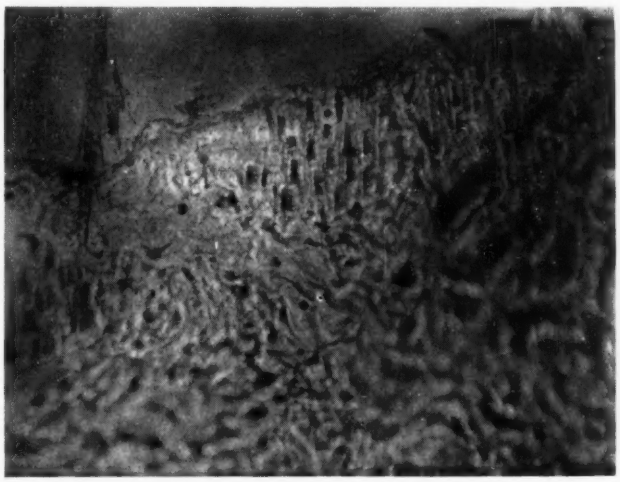

Fig. 1.—Section of skin of a rabbit treated with a metallic mercury cream. The metal can be seen within the stratum disjunctum and in the cutis.

In general, extreme precaution should be taken to insure the freedom from mercury of all equipment. The microtome knife in particular, should be inspected frequently for adherent mercury. Dusts are sometimes troublesome, unless sections are kept covered whenever practicable. It is more conservative practice to withhold a positive identification of mercury unless at least some of the spheres can be seen within the substance of the section, or unless microchemical proof is undertaken.

TYPICAL FINDINGS

Metallic Mercury.—In our experiments, metallic mercury seldom occurred free in the body tissues. We have confirmed Zwick's observation that it may be seen in the subcutis following percutaneous inunction.[6] As he noted, the globules gradually lose their gloss and become roughened as though in process of chemical change. In in vitro experiments we noticed that a similar phenomenon takes place. When mercury is placed in physiologic saline, the droplets gradually lose their shininess. A grayish film appears. Calomel forms and the solution becomes alkaline. It appears probable that the tendency of mercury to react with components of the circulating fluids is responsible for the apparent failure to appear in the organs in metallic form.

We have further confirmed Zwick's finding that mercury globules are easily displaced during sectioning. We know of no certain remedy for this, but we repeat the suggestion that thick sections (15 microns) be used. Zwick thought to minimize the error, or at least to have it operate in his favor, by sectioning from within out. To this may be added the precaution of lowering the section on the reverse stroke of the microtome, so that the globules are not displaced backwards by the blade.

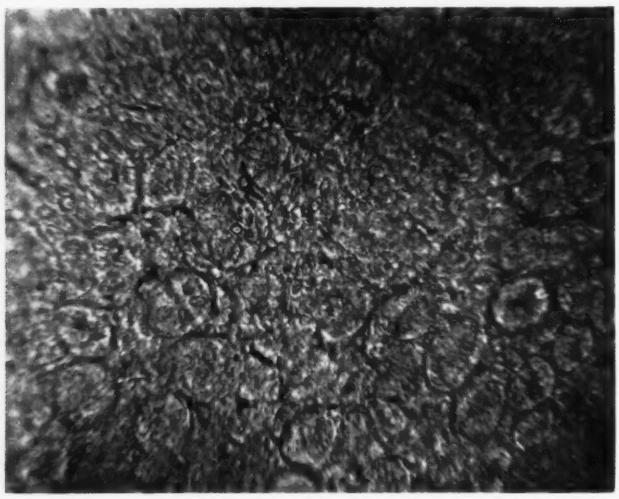

Fig. 2.—Section of kidney of a mouse injected intravenously with mercurous acetate showing mercury within the tubules.

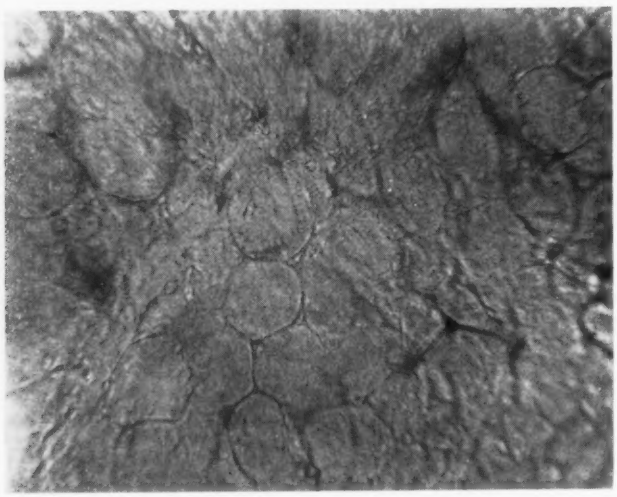

Fig. 3.—Section of kidney of a mouse injected intravenously with mercuric acetate showing mercury within the tubules and interstitially.

Fig. 1 shows the appearance and distribution of metallic mercury in the skin twenty-four hours after inunction from a vanishing cream vehicle.[9]

Mercurous Mercury.—Within a few minutes after the intravenous injection of mercurous acetate in laboratory animals (rats, rabbits, guinea pigs), both

mercurous mercury and mercuric mercury are found in the parenchymal cells and endothelial cells of the kidney. The latter form is preponderant. Even plain whole blood appears to have the capacity to oxidize mercurous salts to the mercuric form in vitro with great rapidity. We do not know what albuminates or complexes may form in the process, but the evidence that oxidation has taken place is based on the specificity of the thioglycollic acid reaction. It may be that subsequent metabolic or excretory processes ultimately lead to a reduction of valency, but the first effect is certainly one of oxidation. Fig. 2 shows mercurous mercury in the kidney of a mouse which had been poisoned by mercurous acetate.

Mercuric Mercury.—It was expected from the preceding observations that the divalent derivatives of this metal would appear in the liver and kidney as mercuric mercury. This proved to be the case. Systematic histochemical examinations have not been carried on over a long enough period of time to permit comment as to possible ultimate changes in valency of mercuric mercury in the body. Such a study might be of value in elucidating the questions of toxicity and excretion.

Fig. 3 shows the characteristic distribution of mercuric mercury in a mouse killed with mercuric acetate.

SUMMARY

Histochemical methods for the determination of mercury have been reviewed and some modifications suggested.

The use of thioglycollic acid for the histochemical differentiation of mercurous and mercuric mercury has been suggested and a method of procedure has been described.

We should like to thank Dr. S. W. Lee, Mr. Thomas Maren, Dr. Franz Herrmann, and Dr. Marion B. Sulzberger for constructive criticism and comment.

REFERENCES

1. Christeller, Erwin, and Sammartino, Rodolfo: Ueber den histochemischen Nachweis des Quecksilbers in den Organen, Ztschr. f. d. ges. exper. Med. **60**: 11, 1928.
2. Feigl, Fritz: Qualitative Analysis by Spot Tests, New York, 1939, Nordeman Pub. Co., pp. 22-23.
3a. Justus, J.: Die Aktion des Quecksilbers auf das syphilistiche Gewebe. 2 Mitteilung. Arch f. Dermat. u. Syph. **60**: 465, 1905.
3b. Justus, J.: Ueber Quecksilbervergiftung, Arch. f. Dermat. u. Syph. **75**: 203, 1905.
4. Almkvist, Johann: Experimentelle Studien über die Lokalisation des Quecksilbers bei Quecksilbervergiftung, Nord. med. Ark. **2**: 1, 1903.
5. Lombardo, C.: La micro- ed isto-chimica nella ricerca tossilogica del mercurio. Arch. di farmacol. sper. **7**: 8, 1908.
6. Zwick, Karl G.: A Microscopic Study of Mercury Absorption From the Skin, J. A. M. A. **83**: 1821, 1924.
7. Emich, Friedrich: Microchemical Laboratory Manual, New York, 1932, John Wiley & Sons, p. 86.
8. Claesson, P.: Ueber Thioglycolsäure, Ann. **187**: 118, 1877.
9. Maren, T. H., and Edwards, B. B.: Studies in the Pharmacology of Mercury. I. Water Soluble Dispersion of Mercury, J. Am. Pharm. A. (Scient. Ed.) **32**: 255, 1943.

LABORATORY METHODS

GENERAL

A SIMPLE MICROMETRIC APPARATUS FOR DETERMINING THE SEDIMENTATION RATE OF THE ERYTHROCYTES*

JULIAN L. ROGATZ, M.D., NEW YORK, N. Y.

SINCE Fahraeus first noted the significance of erythrocytic sedimentation in blood plasma, in 1918, this apparently simple phenomenon has been studied with increasing interest. Its usefulness in determining the presence or absence of infection in the body has been amply verified. Our present understanding of the erythrocytic sedimentation rate, its mechanism, behavior and significance in various diseases, has been admirably summarized by Cutler.[1]

Though the test has been generally accepted and is in constant daily use, there is no agreement as to a suitable apparatus for measuring the rate of settling of the red cells. Unwieldy arrangements with long tubes of wide bore, requiring comparatively large amounts of blood drawn from a vein, have given way to simpler and more practical devices utilizing tubes, or even pipettes, with much narrower bores, requiring not more than a drop of blood easily drawn from the tip of a finger; this is a great advantage in pediatric practice, permitting serial tests with little or no complaint from young patients.

There have been many of these micromethods, which have proved reasonably accurate. The technique usually requires the mixing of blood and anticoagulant separately before placing into the sedimentation pipette. In 1938 I compared what seemed the simplest of these methods, that described by Smith in 1936,[2] with the highly accurate Cutler macromethod and found it equally accurate.[3] Smith feels that in tubes of narrower bore than 2.5 mm. capillarity with its inherent drawbacks cannot be avoided. Thus his remains the only tube available in which capillarity does not occur and in which the figures can be compared directly with a standard venipuncture method. Though the merit of Smith's method may not be denied and its technique is easily mastered, it presents certain objections which militate against its general use by the practitioner and which must be eliminated in the ideal apparatus.

I referred at that time to the Landau modification of the early Linzenmeier-Raunert technique,[4] which seemed to fulfill most of the requirements of an ideal test, i.e., the drawing and mixing of blood and anticoagulant, and the sedimentation of the cells took place in one pipette, thereby eliminating all unnecessary,

*From the Abraham Jacobi Division for Children of the Lenox Hill Hospital, Service of Dr. Jerome S. Leopold.
Received for publication, May 30, 1943.

intervening steps. Careful sealing of the tip of the pipette to prevent loss of the contained specimen in the vertical position seemed a problem. A capillary pipette, similar to that used for the counting of white cells, was used. The column of blood was controlled by a "check syringe" device, which was fitted over the pipette and removed before setting into the rack. The gadget seemed complicated and was not available in this country.

During the past year, however, the Landau apparatus was manufactured locally,* and the "check syringe," on which the usefulness of the method depends, was simplified into an "aspirator" consisting of a metal screw and collar, which fits into a small piece of thick rubber tubing, the whole device being no more than two inches long and one-half inch wide.

The utter simplicity of this apparatus, which consists of only three parts, a pipette, an aspirator, and a rack, and permits of no further simplification of the sedimentation rate technique, impelled me to study its accuracy. I decided to use the proved Smith-Cutler tube as a control. Cases were taken at random as they entered the wards, usually older children because of the cooperation necessary for the drawing of the somewhat larger amounts of blood required for both tests, performed simultaneously. The results of the study on 100 cases, comprising 200 tests and deemed adequate, are presented in the accompanying table (see Table I).

APPARATUS

The Landau microsedimentation apparatus (see Fig. 1) consists of:

1. A capillary pipette 12.5 cm. long with a capillary bore 1 mm. in diameter, expanded into a bulb or mixing chamber 3 cm. from the upper end. Markings are etched at 12.5 mm. and 62.5 mm. from the lower tip, providing for a column of blood 50 mm. long and 1 mm. wide. The intervening space is graduated in millimeters.

2. An "aspirator" consisting of a metal screw with a milled head and its collar, 3.5 cm. long, which fits snugly into a piece of thick rubber tubing 4.5 cm. long. When screwed down, the entire length of the aspirator is 5.5 cm. Turning the screw in either direction controls the position of the column of blood in the pipette, over which it is fastened. When the screw is stationary, the column remains fixed.

3. A metal rack of simple construction with a rubber-cushioned floor and an adjustable rubber-cushioned cap. A hidden spring device in the floor permits depression of the base and holds the pipette vertically and hermetically sealed.

Control apparatus: the Smith micromethod described in a previous study.[2]

PROCEDURE

The upper end of the Landau pipette is fit into the rubber end of the aspirator, which is screwed tightly down. Holding the pipette in the palm of the hand, daggerlike (see Fig. 2), so that the thumb and the index finger control the screw, 5 per cent sodium citrate is drawn up into the pipette by revolving the screw, to the 12 mm. mark. A droplet of blood is then drawn from

*By the Clay-Adams Co., Inc., 44 East 23rd Street, New York City.

the pricked finger, as in a blood count, until the column reaches the 62.5 mm. mark, avoiding air bubbles by keeping the tip of the pipette in constant contact with the blood as it is expressed. Wipe the tip of the pipette clean to prevent later clotting of the blood at this point. The blood and citrate are thoroughly mixed by drawing the column into the ampula or chamber, stopping the lower meniscus a few millimeters below the opening of the chamber to avoid air bubbles. The column of blood is alternately depressed and raised, thus emptying and filling the chamber about seven times to insure proper mixing. The column is finally screwed down until the upper meniscus reaches the mark just below the mixing chamber. The lower tip of the pipette is pressed into the rubber floor of the rack while disengaging the aspirator. The upper end of the pipette is placed beneath the cap of the rack, where it will be held snugly and vertically. After standing for one hour, the millimeter level to which the red cells have fallen is read. The drawing and mixing of blood prior to the beginning of sedimentation should not take more than thirty seconds. The pipette should be cleaned with alcohol and ether after use.

The normal figures reported by Landau were 1 to 6 mm. for men and children under two years; 1 to 9 mm. for women and children over two years; 10 to 15 mm. were considered slightly increased values; 16 to 30 mm. moderately increased; over 30 mm. high. In general I think it sufficiently accurate to consider values under 10 mm. at the end of one hour normal and those up to 15 mm. slightly increased or "high normal" beyond which the rate has increasing significance.

COMMENT

Rapid sedimentation is dependent on the formation of red cells into large clumps or rouleaux, induced by alterations in the plasma, mainly an increase in fibrinogen, which results from the accumulation in the blood stream of certain products of tissue destruction during infections, certain intoxications, malignancy, etc. Cutler thought this change in the plasma proteins plays its predominant role in rouleaux formation, probably through surface dehydration or change in water balance on the surface of the red cells. Globulin, albumin, cholesterol, and electrolytes are involved to a lesser extent.

Smith contended that the influence of capillary exerted within the lumen of the narrow tube and the tendency to clot formation were inherent drawbacks in the pipette methods, but Morrison[3] indicated that the length of the tube used does not affect rouleaux formation, nor does the diameter as long as the tube remains perpendicular. Acceleration of sedimentation in tubes deviating from the perpendicular is dependent on the relative increase in vertical pathways or columns of falling cells. The smaller the diameter of the tube, the greater will be the relative increase in vertical pathways. Though Smith feels the capillarity present in tubes of less than 2.5 mm. bore a disadvantage, this has not proved sufficient in practice to materially affect the accuracy of the pipette method. Clotting of blood in pipettes happened but twice in this series and is therefore not a major objection.

The results obtained in this study correspond satisfactorily in all but four cases, those numbered 37, 42, 75, and 81. In each of these the rate in the Landau

pipette was well within normal limits while the Smith reading was slightly increased. Thus, in Case 37, there was a tonsillectomy in an apparently well child (the Smith reading was 15 mm. at the end of one hour while the Landau reading was 7 mm.). In Case 75, that of a child with purulent otitis media, the

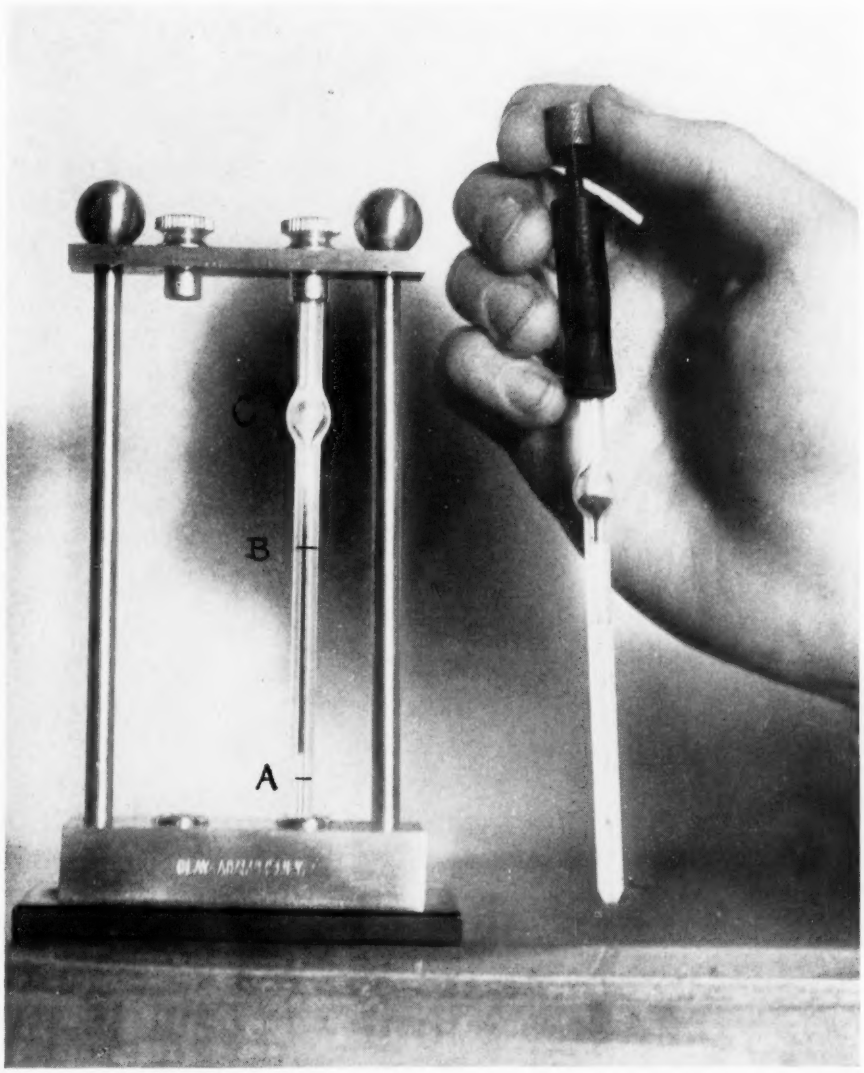

Fig. 1.—Showing Landau micrometric apparatus for sedimentation of the erythrocytes, consisting of metal rack for two pipettes, with one pipette in place, and the rubber aspirator, attached to the other pipette, held in hand. Sodium citrate 5 per cent is drawn to the 12.5 mm. mark (*A*) and blood is then drawn up to the 62.5 mm. mark (*B*), after which the column is drawn into the mixing chamber (*C*). Note specimen of blood in chamber of the pipette with aspirator and note that blood is not drawn completely into the chamber, thus avoiding air bubbles. The pipette in the rack contains a sedimenting column of blood.

Smith reading of 15 mm. seemed more related to the clinical state than the corresponding Landau value of 4 mm. The most marked discrepancy occurred in Patient 81, recovering from an acute tonsillitis, whose rate according to the Smith method was 17 mm. and only 7 mm. by the Landau method.

In the remaining 96 cases the figures corresponded favorably. In some, the high values were somewhat higher in the Landau pipette, while in others

Fig. 2.—Showing method of holding pipette with aspirator attached as blood is withdrawn from tip of a finger.

this was true of the Smith-Cutler tube. The variations, as high as 18 mm. in one case, averaged 7 mm. in 33 patients in whom the higher value appeared in the Landau tube. In 18 cases the situation was reversed, the higher values appear-

TABLE 1

TABLE OF CASES

CASE NUMBER	AGE YEARS	DATE	DIAGNOSIS	SMITH MICROMETRIC MM.		NEW LANDAU MICROMETRIC MM.	
				½ HR.	1 HR.	½ HR.	1 HR.
1	10	9/ 1/42	Tonsillectomy	1	3	2 sharp	4 sharp
2	8	9/ 1/42	Tonsillectomy	1	2	2 sharp	3 sharp
3	9	9/ 4/42	Convalescent bronchitis	8	16	2 blurred	25 sharp
4	7	9/ 4/42	Convalescent "grip"	13	20	? blurred	38 sharp
5	11	9/ 5/42	Acute sinusitis	18	24	10 sharp	25 sharp
6	10	9/ 5/42	Convalescent pneumonia	6	16	2 blurred	26 sharp
7	11	9/13/42	Melanoma ? of eye	4	9	1 blurred	4 sharp
8	8	9/13/42	Post tonsillectomy	10	18	2 blurred	28 sharp
9	9	9/14/42	Accessory scaphoids	2	4	1 sharp	2 sharp
10	8	9/15/42	Cleft palate	2	6	2 sharp	3 sharp
11	8	9/15/42	Convalesc. rheumatic fever	1	5	1 sharp	6 sharp
12	11	9/16/42	Convalescent bronchitis	5	12	2 blurred	5 blurred
13	6	9/16/42	Convalescent pneumonia	9	16	1 blurred	20 blurred
14	4	9/17/42	Acute appendicitis ?	7	18	10 blurred	17 sharp
15	8	9/17/42	Convalesc. rheumatic fever	2	6	1 sharp	3 sharp
16	5	9/24/42	Catarrhal otitis media	24	29	1 blurred	41 sharp
17	10	9/28/42	Convalescent "grip"	4	8	1 sharp	3 sharp
18	9	9/28/42	Asthmatic bronchitis	7	17	2 sharp	20 sharp
19	9	9/29/42	"Grip"	5	23	20 sharp	28 sharp
20	6	9/29/42	Convalescent bronchopneumonia	8	17	1 sharp	23 sharp
21	8	10/ 6/42	Convalescent "grip"	6	18	2 blurred	26 sharp
22	6	10/ 9/42	Acute catarrhal jaundice	8	16	2 sharp	29 sharp
23	9	10/ 9/42	Lobar pneumonia	13	18	8 sharp	22 sharp
24	9	10/15/42	Convalescent bronchopneumonia	8	15	7 blurred	23 sharp
25	7	10/15/42	Aphthous stomatitis	7	14	2 sharp	10 sharp
26	9	10/23/42	Tonsillectomy	2	5	2 sharp	4 sharp
27	6	10/23/42	Convalescent "grip"	6	12	4 sharp	12 sharp
28	5	10/23/42	No pathology apparent	6	12	4 sharp	12 sharp
29	9	10/23/42	Acute peritonitis	13	23	13 blurred	22 sharp
30	8	10/28/42	No pathology apparent	14	18	10 blurred	24 sharp
31	10	10/28/42	Convalescent "grip"	5	10	3 sharp	5 sharp
32	14	11/ 4/42	No pathology apparent	2	5	3 sharp	6 sharp
33	4	11/ 4/42	Convalescent "grip"	4	9	3 sharp	6 sharp
34	10	11/ 5/42	"Grip" ?	4	12	2 blurred	15 sharp
35	8	11/ 5/42	Convalescent "grip"	12	20	12 blurred	23 sharp
36	9	11/ 6/42	Appendicitis ?	8	15	2 blurred	13 blurred
37	6	11/ 6/42	Tonsillectomy	8	15	2 sharp	7 blurred
38	11	11/ 6/42	Pneumonia	21	28	6 blurred	20 blurred
39	7	11/10/42	"Grip"	10	18	2 sharp	13 blurred
40	6	11/10/42	Acute bronchitis	6	12	3 sharp	9 sharp
41	8	11/13/42	Tonsillectomy	4	10	3 sharp	4 sharp
42	1	11/13/42	Pertussis	12	21	6 blurred	10 blurred
43	10	11/18/42	Tuberculous hip ?	4	8	2 sharp	4 sharp
44	7	11/18/42	"Grip"	5	12	2 sharp	11 blurred
45	5	11/19/42	Convalescent pneumonia	13	20	3 blurred	13 blurred
46	4½	11/19/42	Gastroenteritis	5	10	2 blurred	5 sharp
47	12	11/27/42	Virus (atypical) pneumonia	5	10	4 sharp	9 sharp
48	5	11/27/42	No pathology apparent	3	10	1 sharp	3 sharp
49	8	12/ 2/42	Acute bronchopneumonia	22	27	5 blurred	20 blurred
50	9	12/ 3/42	Fracture of humerus	2	8	2 sharp	3 sharp
51	8	12/ 3/42	Acute bronchopneumonia	29	23	19 sharp	21 sharp
52	5	12/ 3/42	Acute mastoiditis	34	35	27 sharp	30 sharp
53	7	12/ 3/42	Inguinal hernia	8	18	1 sharp	2 sharp
54	8	12/ 5/42	T.B. cervical adenitis	5	10	1 sharp	4 sharp
55	12	12/ 5/42	Convalesc. appendectomy	10	18	2 blurred	28 sharp
56	8	12/ 7/42	Upper respiratory infection	14	22	15 blurred	33 sharp
57	9	12/ 7/42	Hemangioma	6	12	1 sharp	2 sharp
58	12	12/ 8/42	Convalesc. appendectomy	3	8	1 sharp	3 sharp

TABLE I—CONT'D

CASE NUMBER	AGE YEARS	DATE	DIAGNOSIS	SMITH MICROMETRIC MM. ½ HR.	SMITH MICROMETRIC MM. 1 HR.	NEW LANDAU MICROMETRIC MM. ½ HR.	NEW LANDAU MICROMETRIC MM. 1 HR.
59	5	12/ 8/42	Chronic mastoiditis	5	17	1 sharp	13 blurred
60	10	12/10/42	Acute tonsillitis	15	26	2 sharp	26 sharp
61	8	12/10/42	Convalescent pneumonia	20	25	12 sharp	24 sharp
62	8	12/15/42	Chronic cardiovascular disease	15	23	8 blurred	23 sharp
63	6	12/15/42	Convalesc. C.S. meningitis	2	9	1 sharp	10 sharp
64	6	12/21/42	Tuberculous hip	8	15	2 sharp	20 sharp
65	8	12/21/42	Tuberculous pleurisy	6	13	1 sharp	9 sharp
66	6	12/21/42	Convalescent meningitis	3	9	1 sharp	2 sharp
67	8	12/21/42	Chronic cardiovasc. disease	3	9	1 sharp	2 sharp
68	9	12/31/42	Convalescent pneumonia	26	28	39 sharp	42 sharp
69	6	12/31/42	Acute tonsillitis	17	22	10 blurred	20 sharp
70	8	12/31/42	Tuberculous pleurisy	2	8	1 sharp	7 sharp
71	7	12/31/42	Acute bronchopneumonia	7	24	1 sharp	36 sharp
72	6	1/ 7/43	Pleurisy	19	23	2 blurred	25 blurred
73	3	1/ 7/43	Eczema	19	24	15 blurred	24 sharp
74	2	1/ 8/43	Acute tonsillitis	27	29	21 sharp	28 sharp
75	6	1/ 8/43	Acute otities media purulenta	9	15	2 sharp	4 sharp
76	8	1/11/43	Wrist injury	4	7	4 sharp	5 sharp
77	8	1/11/43	Cervical adentitis (acute)	23	27	35 sharp	38 sharp
78	6	1/14/43	Acute tonsillitis	18	22	10 sharp	16 sharp
79	8	1/14/43	Acute catarrhal otitis media	25	28	32 sharp	38 sharp
80	8	1/15/43	Convalescent pleurisy	2	5	1 sharp	2 sharp
81	6	1/15/43	Convalescent acute tonsillitis	8	17	1 sharp	7 blurred
82	10	1/16/43	Acute mastoiditis	12	21	15 blurred	26 sharp
83	6	1/16/43	T.B. cervical adenitis	3	5	2 sharp	4 sharp
84	14	1/18/43	Tuberculous hip	22	30	15 sharp	18 sharp
85	10	1/18/43	Fracture of elbow	1	3	1 sharp	2 sharp
86	5	1/19/43	Chronic cardiovalv. disease	2	6	2 sharp	5 sharp
87	4	1/19/43	Acute tonsillitis	14	23	27 sharp	38 sharp
88	13	1/21/43	Chorea	2	4	2 sharp	3 sharp
89	6	1/21/43	Clubbed feet	9	17	11 blurred	25 sharp
90	6	1/25/43	"Grip"	2	4	1 sharp	2 sharp
91	12	1/25/43	Convalescent rheumatic fever	2	6	1 sharp	2 sharp
92	8	1/26/43	"Grip"	5	15	1 blurred	29 sharp
93	4½	1/26/43	"Grip"	4	14	2 sharp	13 blurred
94	4½	1/28/43	Acute ethmoiditis (acute hemolytic anemia)	31	35	41 sharp	44 sharp
95	2	1/28/43	Tonsillectomy	6	20	20 blurred	33 sharp
96	7	1/28/43	Convalescent meningitis	30	34	37 sharp	40 sharp
97	5	1/28/43	Convalescent appendicitis	2	8	1 sharp	11 sharp
98	14	1/28/43	Tuberculosis of Spine	14	27	26 sharp	39 sharp
99	8	1/28/43	"Grip"	3	8	1 sharp	3 sharp
100	13	2/12/43	Hypertension	7	14	6 sharp	12 sharp

ing in the Smith-Cutler tube, the greatest variation being 12 mm., the average 5 mm. These differences are not great when it is considered that they occurred in sedimentation rates which were very rapid. They are, therefore, not significant.

After reading a number of sedimentation tests, it soon becomes apparent that there may be two types of reaction. The separation of red cells from plasma is usually indicated by a sharp demarcation. Frequently, however, it may be diffuse or blurred. This happens occasionally in other tubes with larger bores, as it did a number of times in the Cutler tubes. It is most likely to occur early in

a test the sedimentation of which proves rapid. There is a gradual tapering of color from above downward, the pale plasma deepening into the pinkish color of sinking erythrocytes, which merges into the deep red of packed cells. By the end of an hour the blurring was usually replaced by a sharp line. In only nine cases of this series did blurring persist after one hour. In Case 36 it cleared in seventy minutes.

As the cells pack, the diffused area covers only a few millimeters and the line of probable demarcation can be easily estimated within one or two millimeters, which is sufficiently accurate for all practical purposes. If a reading is deemed unsatisfactory, it is possible to reattach the aspirator to the pipette, start drawing the column into the mixing chamber before releasing pressure on the floor of the rack, and allow the cells to sediment anew, provided the blood has not clotted in the pipette. The second reading may be more satisfactory.

SUMMARY

The new Landau microsedimentation apparatus is described, and its reliability is demonstrated in a series of 100 cases, in which the proved Smith-Cutler method was used as a control. The apparatus and technique have been reduced to their simplest possible form by successfully combining the several necessary steps, namely, the drawing and mixing of blood and anticoagulant and the sedimenting of the red cells, into one uninterrupted procedure accomplished in one pipette. Only three pieces of apparatus of the simplest possible construction are required: the pipette, an aspirator, and a rack. Not more than $\frac{1}{20}$ c.c. of blood is used, and the time required from puncture of the finger to the beginning of sedimentation is about thirty seconds. The set is so compact that it may be placed in a small corner of one's bag. No previous experience or unusual skill is necessary for the efficient use of this method. A test of acknowledged significance thus becomes universally available to practitioner and specialist alike, equally useful at the bedside or in the laboratory.

CONCLUSION

The new Landau erythrocytic microsedimentation test fulfills the requirements of the ideal method. It is deemed worthy of further trial. Its reliability having been adequately demonstrated, it is suggested that this technique and apparatus may help to establish the desired standardization of the sedimentation rate test.

REFERENCES

1. Cutler, J. W.: Present Concept of Erythrocyte Sedimentation Rate, New Internat. Clin. **3**: 62, 1940.
2. Smith, C. H.: A Method for Determining the Sedimentation Rate and Red Cell Volume in Infants and Children With the Use of Capillary Blood, Am. J. M. Sc. **192**: 73, 1936.
3. Rogatz, J. L.: Microsedimentation of the Erythrocytes in Infants and Children, Am. J. Dis. Child. **56**: 1037, 1938.
4. Landau, A.: Microsedimentation, Am. J. Dis. Child. **45**: 694, 1933.
5. Morrison, L.: Observations on Blood Sedimentation Mechanism, Am. J. Clin. Path. **11**: 578, 1941.

PORTABLE APPARATUS FOR ESTIMATING THE PERIPHERAL BLOOD FLOW IN SHOCK*

HERBERT S. WELLS, M.D., AND JOHN J. THOMPSON, A.B., WINSTON-SALEM, N. C.

SKIN pallor in shock suggests that the peripheral blood flow is reduced, but it is difficult to estimate the degree of this reduction from the intensity of the pallor or other clinical signs. The one series of measurements carried out on human cases[1] indicates the usefulness of quantitative information on blood flow for gauging the degree of developing circulatory failure and the response to transfusion. The direct plethysmographic method, however, commonly used to measure blood flow, requires cumbersome equipment not easily adapted to studies at the bedside or in the operating room or casualty station. The method to be described, while not so accurate as the more elaborate equipment of the physiologic laboratory, is practical in that it gives consistent readings which serve well for comparative studies, and because the apparatus is portable and easy to operate.

The apparatus is essentially a small air plethysmograph consisting of a cuff, 1¼ inch wide, which supports a thin rubber bag against the skin. As the limb swells, following quick inflation of a centrally located sphygmomanometer cuff, air is expelled from the cuff bag, whence it passes to one end of a horizontal pipette (1 c.c. calibrated in 0.1 c.c. units) containing a droplet of water, the movement of which measures the rate of swelling of the limb. The distal end of the pipette connects to a second rubber bag upon which a lead weight floats. This constant pressure or "reservoir" bag (*R B* in Fig. 1) maintains a nearly uniform pressure of 6 cm. water in the whole system, which is sufficient to inflate the cuff for contact with the skin. Movement of the water drop is timed by counting swings of a pendulum whose period is 0.5 second. The number of pendulum swings for successive 0.1 c.c. excursions of the water drop are recorded. These data, together with the volume of the limb enclosed by the cuff, which is indicated by markings on the cuff, enable calculation of the blood flow in c.c. per 100 c.c. of limb per minute. An alignment chart is used, in practice, to facilitate the calculations.

It has been found that the cuff bag need not completely surround the limb. A bag 7 inches long, made from ¾ inch Penrose drainage tubing and cemented to a cuff 15 inches in length, may be applied to a limb segment of volume up to 250 c.c. For use on the leg a longer cuff and bag may be required.

Several forms of the apparatus have been designed, but the most compact and readily portable type, shown in the upper sketch of Fig. 1, has proved most practical. All necessary equipment except the sphygmomanometer (aneroid

*From the Laboratories of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N. C.

Aided by a grant from the Council on Physical Therapy of the American Medical Association.

Received for publication, April 27, 1943.

type) is contained in a box which is 4¼ x 9 x 1¼ inches when closed. The traps, *T* in the diagram, which prevent loss of the water drop, enable the box to be carried in any position. The pipette is mounted on a strip of wood, which may be turned forward, allowing the water traps to lie in a horizontal position so the cover may be closed. The cuff, the metal paper clip (not shown) used for holding the cuff in place on the limb, and a tourniquet for the wrist (18 inches of 1 inch Penrose tubing) may be placed in the box when not in use. A short thermometer, suitable for measurement of the skin temperature, is held in the upper part of the metal clip shown inside the cover of the box. The lower part of this clip serves to clamp the pendulum when not in use.

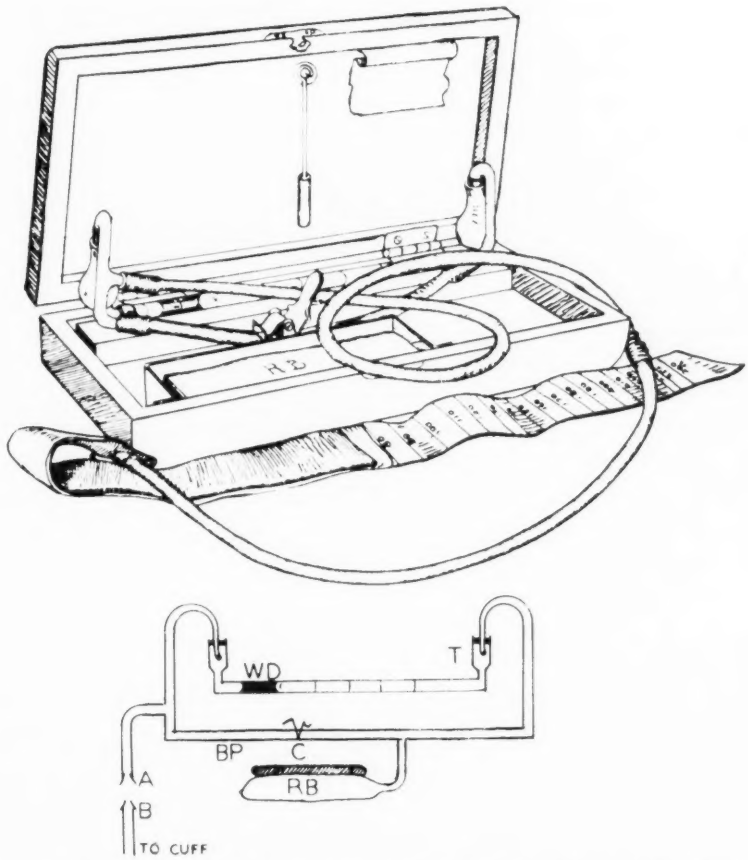

Fig. 1.—Drawing and diagram of blood flow apparatus. *W D*, water drop in 1 c.c. pipette; *T*, water traps at each end of the pipette; *A* and *B*, metal connectors on tubing to the cuff bag; *B P*, by-pass tube, which is opened or closed by clamp, *C*; *R B*, reservoir bag, 4 inches long, similar to the cuff bag, and having a lead weight of 150 Gm. "floated" upon it.

A by-pass, *B P*, which is closed by the clamp, *C*, during the determination, is opened to allow air to pass between the cuff bag and the reservoir bag during inflation of the bag system or during movements of the patient which might otherwise cause the water drop to be blown out of the pipette. To inflate the bag, the cuff is first fitted snugly, but not tightly, to a segment of the arm or leg,

which should be as nearly circular in cross section as possible. The metal tubing connectors, *A* and *B*, are separated. The operator blows into *A* until the reservoir bag, *R B*, is inflated. Pinching off the rubber tube above *A*, he then connects *A* and *B*. When the tube is released, air from *R B* passes back into the cuff bag.

Spontaneous variations of blood flow are reduced to a minimum, if the circulation to the hand is occluded by a tight tourniquet of Penrose tubing wrapped tightly just above the wrist.

The accuracy of the method was tested by comparison of simultaneous recordings, which measured the swelling rates following venous occlusion of (a) the whole forearm inserted in a water-jacketed air plethysmograph and (b) of a segment of this forearm, encircled by the cuff plethysmograph. For recording purposes a slightly modified form of the apparatus was used in which the reservoir bag system was replaced by a constant pressure water reservoir, from the open end of which air was displaced at atmospheric pressure to a volume recorder. A second recorder registered the displacement of air from the larger plethysmograph. The recorders consisted of soap films formed across the ends of horizontal glass tubes. Images of the bubbles, which increased in size and approached each other as air entered from the respective plethysmographs, were projected at convenient magnification across the horizontal slit of a 12 cm. recording camera. Time in seconds, calibration lines for the two recorders, and a mercury manometer record of the pressure applied to the collecting cuff were also photographed. Soap film recorders (first used in this laboratory) respond to pressure changes of less than 0.005 mm. H_2O; and errors of amplitude, time lag, and maximum rate of response during tests, with simple harmonic volume changes, are imperceptible up to a frequency of 3 to 5 cycles per second. The natural frequency of the larger recorder was 12 to 15 per second.

The cuff plethysmograph, as tested in this manner on 5 normal subjects (23 determinations) gives values of the blood flow, per unit volume of limb, which are regularly low. The mean error is -28 ± 11.3 per cent, the range being from –10 to –47 per cent.

It is evident that the absolute values obtained cannot be relied upon to give better than an estimate of the actual blood flow. The readings, however, are consistent and reproducible, and comparative measurements are reliable. Furthermore, the variability (standard deviation as per cent of the mean) of readings on normal subjects is not greater than is the case for more accurate methods. The mean of measurements on 54 normal subjects was 2.65 ± 0.96 c.c. per 100 c.c. per minute. All subjects were comfortably warm, at room temperatures between 21° C. and 30° C. and with forearm skin temperatures from 29° C. to 34° C.

In most cases it makes no difference whether the cuff is placed around the upper, middle, or lower third of the forearm; the same value for blood flow is obtained in each case. This indicates a remarkable uniformity (from which, however, some subjects show considerable departures) of the swelling of all portions of the limb. It seems evident that the chief cause of the error of the cuff plethysmograph is referable to the slight compression of blood vessels lying be-

neath it rather than to the location of the cuff on a portion of the arm which swells at less than the average rate for the limb as a whole.

During the past year the method has been tried in clinical practice in several hospitals on over a hundred patients, including a number suffering from various forms of circulatory failure (cardiogenic, vasogenic, hematogenic types). The cases, briefly reported in Table I, may suffice to indicate the low levels of peripheral blood flow found in hematogenic shock. The increase of the flow to or toward normal levels, following transfusion, is noteworthy. The tendency of the blood flow to vary inversely with the hematocrit readings in Case 4, is of particular interest, since a blood flow determination may be made in a fraction of the time required for a hematocrit. In general, we may say that a blood flow value of less than 1.0 c.c. per 100 c.c. per minute, which persists after pain, nausea, cold, or other confusing factors have been eliminated, is highly suggestive of peripheral circulatory failure of the hematogenic type. Further clinical studies will of course be necessary to determine the range of usefulness of the method.

TABLE I

CASE NO.	TIME AFTER INJURY	BLOOD PRESSURE	HEMATOCRIT	BLOOD FLOW	REMARKS
	hours	mm. Hg	per cent cells	c.c. per 100 c.c. per minute	
1	5	96/60	–	0.8	Gastroenterostomy followed by profuse hematemesis, seven hours. Transfusions, whole blood, 1500 c.c. to tenth hour; 1000 c.c. twenty-first hour.
	10	70/50	–	0.4	
	21	120/76	–	2.4	
2	1	50/?	–	0.5	Stab wound of thorax. Transfusion 500 c.c. whole blood first to fifth hours. Bleeding not controlled until third hour.
	3	68/?	–	0.6	
	5	110/74	–	1.1	
3	4	50/?	–	0.7	Post-partum hemorrhage. Transfusion, 800 c.c. glucose, 500 c.c. plasma from fourth to sixth hours.
	5	108/80	–	2.7	
	6	120/82	–	3.6	
4	0.5	–	38.2	–	Severe second degree burn. Received 5300 c.c. plasma during thirty hours.
	6	–	45.2	2.3	
	17	–	58.8	0.6	
	108	–	38.7	2.5	

The blood flow apparatus serves a second useful function in shock cases, for it enables one to determine the systolic blood pressure in those patients (unexpectedly numerous, in our experience) in which the usual methods fail and the pressure is reported as "unobtainable." As the sphygmomanometer cuff pressure is lowered to the systolic level, blood entering the forearm, even if its weak pulsations cannot be heard with the stethoscope or palpated at the wrist, will cause the water drop in the pipette of the blood flow apparatus to move. A very sharp measure of systolic pressure can always be determined in this way.

REFERENCE

1. Freeman, N. E., Shaw, J. L., and Snyder, J. C.: The Peripheral Blood Flow in Surgical Shock. The Reduction in Circulation Through the Hand Resulting from Pain, Fear, Cold, and Asphyxia, With Quantitative Measurements of the Volume Flow of the Blood in Clinical Cases of Surgical Shock, J. Clin. Investigation **15**: 651, 1935.

A RESPIRATORY CHAMBER FOR CHRONIC EXPOSURE TO GASES*

A. T. Miller, Jr., Chapel Hill, N. C.

THE respiratory chamber described in this paper is a modification of the one used by the author in previously reported work (Miller, 1940[1]) on the effects of chronic exposure to carbon dioxide. The apparatus itself has not previously been described. The chamber now in use in this department was designed specifically for studies on chronic carbon monoxide exposure, but is readily adaptable to the study of other toxic gases, including war gases.

The over-all dimensions of the chamber are 11 feet by 7 feet by 6 feet. The actual gas chamber is 8 feet by 7 feet by 6 feet, giving a capacity of 336 cubic feet. The remainder of the capacity is accounted for by the space between the double entrance doors. The chamber will readily accommodate three human subjects, or 12 dogs, or 200 rats.

The chamber is constructed of 24 gauge sheet metal placed over a framework of two by fours. The metal is fastened to the framework with galvanized nails so that the junction between nail heads and sheet metal can be soldered. All seams are soldered and the entire chamber is painted inside and out with heavy floor enamel. Control experiments indicate that a gas mixture placed in the chamber suffers no measureable change in composition during forty-eight hours.

The chamber has a wooden floor elevated four inches from the sheet metal bottom and sealed to prevent trapping of gases between the two. The entrance doors are built of 1½ inch redwood, reinforced by 1 inch angle iron to prevent warping. The door overlaps the door facing 2 inches on all sides, and when the door is closed, it is sealed by a gasket consisting of rubber tubing set in a continuous groove on the inner surface of the door overlap. Each door is closed by two commercial cold room handles which exert enough pressure to collapse the rubber tubing gaskets against the door facing. The double door entrance minimizes changes in gas composition produced by entering and leaving the chamber during the course of an experiment.

Two windows, each 3 feet by 2 feet, are placed in the chamber for illumination and observation. Additional illumination is provided by an overhead light bulb, and an even mixture of gases is maintained by an oscillating fan. Electric wires are led into the chamber through porcelain insulators and are sealed with cement.

A continuous circulation of air through the chamber maintains a normal pressure of oxygen and removes carbon dioxide. As shown in Fig. 1, this circulation is provided by an 8 inch electric fan mounted directly into a funnel-like expansion of the 4 inch tube leading into the chamber. A 4 inch exit tube leads

*From the Department of Physiology, School of Medicine, University of North Carolina. Aided by a grant-in-aid from the Sigma Xi Alumni Research Fund.
Received for publication, May 5, 1943.

to the outside of the building. The gas to be studied is led into the ventilating tube through a side tube as shown in the diagram.

The maintenance of a desired gas concentration in the chamber requires two things: a constant rate of ventilation and a constant rate of flow of the gas into the ventilating tube. Flowmeter tests indicate that an electric fan kept in good working condition maintains a very constant ventilation rate. A constant inflow of gas requires a source of gas under a constant pressure, with adjustment of the rate of flow by suitable reducing valves. Illuminating gas is a convenient source of carbon monoxide in amounts large enough for continuous flow. The low pressure at which this gas is ordinarily delivered and the fluctuations in rate of flow require a system for storing the gas under pressure for delivery to the gas chamber. The details of this system are shown in Fig. 1. The gas flows from the jet into a 50 liter Tissot spirometer. This serves as a reservoir from which gas is compressed into a pressure tank. The compression pump is a Crowell rotary number OD with a maximum pressure rating of 25 pounds and a free air flow of 2 cubic feet per minute. The pump is operated by a ¼ H. P. motor. The pressure tank is equipped with an automatic pressure regulator ("Pressuretrol" type L 404-2, made by the Minneapolis-Honeywell Regulator Company) with a pressure range of 2 to 50 pounds. This pressure regulator is connected to a pump motor through a Colt "Noark" automatic switch. The pressure regulator is set for a cutin pressure of 18 pounds and a differential of 2 pounds, so that gas is always delivered from the pressure tank to the respiration chamber at a pressure between 18 and 20 pounds. The outlet from the pressure storage tank is provided with a gate valve for cutting off the flow completely at the end of an experiment and with a Hoke ammonia control valve for fine adjustment of flow. This valve permits accurate control from full flow down to 5 bubbles per minute. In practice, the concentration of carbon monoxide in the respiration chamber can be controlled within 0.001 per cent during a 6 hour exposure period.

The absolute variation in rate of flow of gas into the tank may be determined as follows. Gas is delivered from the storage tank at a pressure which steadily declines from 20 to 18 pounds over a period of about ten minutes. At this point, the pump cuts on and the pressure is built up to 20 pounds again in about one and one-half minutes. The average delivery pressure then is 19 pounds with a range of ± 1 pound, or 5 per cent of the total pressure. This means that a given rate of flow can be maintained within the limits of ± 5 per cent, and this is proved by the fact that the fluctuations in the flowmeter reading are uniformly within these limits (see Fig. 2). If a still more uniform rate of flow is necessary, it can be obtained either by using a pressure regulator with a smaller differential range or by inserting a constant flow valve (such as those used on oxygen cylinders) between the pressure tank and the respiration chamber.

The operation of the entire gas flow assembly is made completely automatic by providing the bell of the Tissot spirometer with an automatic checking device. The rise of the bell is checked at such a height that the depth of the bell below the water level is greater than the gas pressure in the main, in centimeters of water. The pressure in the bell increases until it equals the main pressure and the flow of gas from the main ceases. Guy ropes attached to the top of the spirometer bell check it at the desired point.

The concentration of carbon monoxide in the respiration chamber may be determined by the usual analytic methods. A great saving of time and effort is made by the use of a carbon monoxide indicator (Mine Safety Appliances Company) which requires only about two minutes for a determination.

Routine determination of the carbon monoxide concentration in the chamber may be obviated in the following way. The flowmeter is calibrated against the carbon monoxide concentration in the chamber throughout the range of concentrations to be used. It should be emphasized that this calibration must be experimental, since the logarithmic relation between flowmeter pressure differential and rate of flow breaks down at rates of flow high enough to produce turbulence. Once the calibration is performed, the desired carbon monoxide concentration is obtained by adjusting the fine control valve to give the proper flowmeter reading. The oxygen and carbon dioxide concentrations should be checked occasionally, although they have never been found outside normal limits in work in this department. The relative humidity is recorded at the end of an exposure period by the wet bulb method. This may be done without entering the chamber by inserting the wet bulb thermometer through a rubber stopper into a flask through which a stream of air from the chamber is drawn by suction. The relative humidity has never been observed to rise above that of the outside atmosphere even with a full complement of experimental animals in the chamber.

If the effects of carbon monoxide are to be studied uninfluenced by other constituents of illuminating gas, these constituents must be removed. Most artificial gas is a mixture of coal gas and water gas. The Chapel Hill city gas has the following percentage composition:

Carbon monoxide	23.4
Hydrogen	36.8
Methane	17.8
Illuminants (unsaturated hydrocarbons)	8.6
Nitrogen	8.5
Carbon dioxide	3.7
Oxygen	1.2
Benzene (not included in analysis furnished by gas company but usually present to the extent of 1 or 2 per cent).	

This is a characteristic composition for manufactured gas, although the relative proportions of the constituents may vary in different communities. Of these, hydrogen, nitrogen, and methane are simple asphyxiants; that is, they are inert gases which act by excluding oxygen from the lungs when present in high concentration. The unsaturated hydrocarbons have anesthetic properties in high concentration (about 60 per cent), but are simple asphyxiants in low concentrations. All of these gases are present in extremely low concentration in the final mixture of air and gas delivered to the chamber (less than 0.2 per cent for the sum of all of them when the final carbon monoxide concentration is 0.05 per cent) so that their effect is negligible and they need not be removed. The final concentration of carbon dioxide under the same conditions is less than that of atmospheric air. Benzene is the only constituent aside from the carbon monoxide which is present in sufficiently high concentration to exert specific effects. The greater toxicity of illuminating gas compared with pure carbon

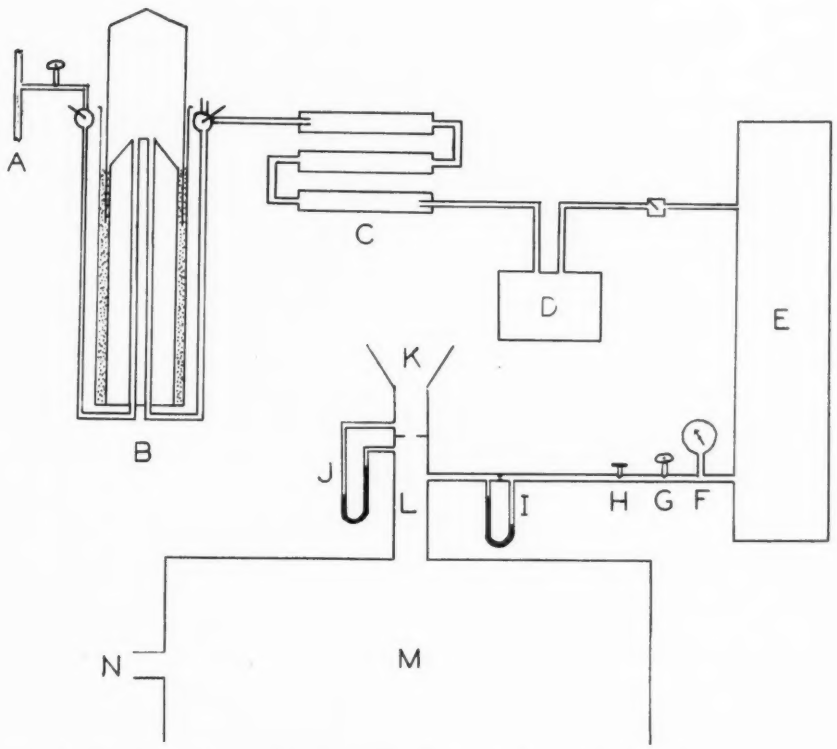

Fig. 1.—Diagram of assembly for controlling gas flow and ventilation. *A*, gas main; *B*, Tissot spirometer; *C*, charcoal absorption tubes; *D*, compression pump; *E*, pressure storage tank; *F*, pressure gauge; *G*, gate valve; *H*, reducing valve; *I* and *J*, flowmeters; *K*, position of ventilating fan; *L*, ventilation tube; *M*, gas chamber; *N*, exit tube of ventilating system. The motor which operates the pump and the automatic pressure control are not shown in the diagram.

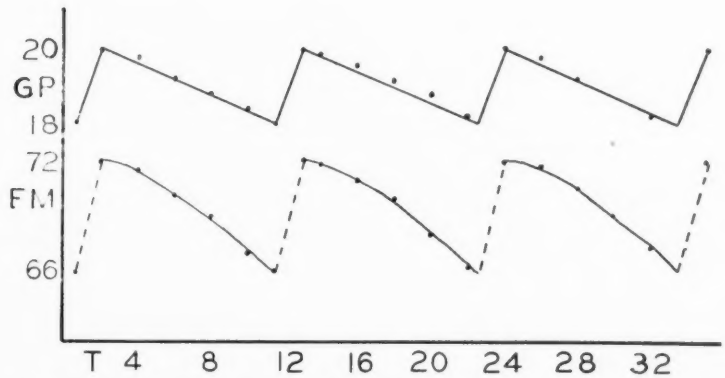

Fig. 2.—Relation between storage tank gauge pressure and rate of flow. Abscissa, time in minutes. Ordinate, *FM* = flowmeter readings in mm. glycerin, *GP* = gauge pressure in pounds.

monoxide is usually attributed to the presence of benzene. This may be removed quantitatively from the illuminating gas by passage through absorption tubes containing activated charcoal, as shown in Fig. 1. The completeness of removal may be tested by bubbling the gas through a test bottle containing a dilute solution of formaldehyde in sulfuric acid (5 c.c. of 40 per cent formaldehyde diluted to 100 c.c. with concentrated sulfuric acid). A reddish-purple color indicates the presence of benzene.

Only slight modifications are required to adapt the chamber to the use of other toxic gases, including war gases. If the gas to be used is lighter than air, no essential modification is necessary, since the gas leaving the exit tube of the chamber is quickly dissipated by dispersion. If heavier-than-air gases or vapors are to be used, the exit tube should lead through a suitable trap for absorption of the gas or vapor. If corrosive gases, such as chlorine, are used, all surfaces with which the gas comes in contact must be painted with one of the resistant paints now commercially available.

SUMMARY

A respiration chamber and gas train for chronic exposure to carbon monoxide are described. The assembly is automatic in operation and requires no attention during an eight-hour exposure period. The slight modifications necessary for adapting the chamber to the use of other toxic gases are described.

REFERENCE

1. Miller, A. T., Jr.: Acclimatization to Carbon Dioxide. A Study of Chemical and Cellular Changes in the Blood, Am. J. Physiol. **129**: 524, 1940.

IMMUNOLOGIC REACTIONS FOLLOWING TYPHUS VACCINATION IN ARMY PERSONNEL*

JAMES H. PARK, M.D.† AND FREDERICK M. OFFENKRANTZ, M.D.‡

THE term typhus as generally used refers to three groups of rickettsial diseases: 1. Classical typhus (which includes Murine typhus) and a louse- or flea-borne group. 2. Rocky Mountain Spotted fever, which is caused by a tick-borne group. 3. Scrub typhus and tsutsugamushi fever, which are mite-borne diseases. The known role of typhus as a decimater of civilian and military populations in wartime is too well known to be commented upon further. The form of typhus which has been responsible for this stigma is the Classical group, epidemic throughout Europe. Because of its importance as a public health measure, the War Department, even prior to the present conflict, began immunization of all military personnel destined to enter potential or actual typhus areas. The form of vaccine employed was the rickettsial preparation grown by Cox in the yolk sac of developing chick embryos. Although of proved protective potency in animals, the only large scale trial of this method of vaccination was during the Spanish Civil War, by Rockefeller Foundation workers. Unfortunately they were unable to remain for a period of time sufficient to evaluate their results. The use of vaccine prepared from *Rickettsia prowazeki* (the causative agent of Classical typhus) by the War Department was made standard for personnel going to the Southwest Pacific area as well as points closer to actual epidemic sites of that form of the disease. In the Australasia zone, however, the Scrub typhus or tsutsugamushi group constitutes the endemic and epidemic form. The causative organism here is the *Rickettsia orientalis.*

It is too early to state with assurance that protection has been afforded those exposed to Scrub typhus by immunization with the agent of the Classical form, and complementing this consideration is the fact that no large bodies of our troops have yet been exposed to the Classical form for estimation of the vaccine efficiency.

The inception of our study lay in the need for immunologic data with regard to typhus vaccination in human beings. The method of such study can only be by employing the Weil-Felix reaction. This procedure involves the use of *Bacillus proteus*, the Somatic (O) variety of the X19 and XK strains. The reaction of the Classical group has been to the OX19, while that of the Scrub typhus group has been to OXK. Emphasis has been laid upon the sharp distinction afforded between the groups by the use of this differential reaction. To us in this zone the problem was twofold; 1. What antibody response was being afforded by use of the vaccine. 2. To ascertain serologically the dilution at which agglutination was diagnostic of Scrub typhus.

*From the Third Medical Laboratory, Advance Section, Base Section 2, APO, Postmaster, San Francisco.

†Major, Medical Corps.

‡Captain, Medical Corps.

Received for publication, May 13, 1943.

This study was conducted upon a series of 507 sera collected at random from patients coming through our laboratory for Kahn testing. The blood was collected by venipuncture directly into sterile Wassermann tubes and clotting permitted at room temperature. The serologic testing was performed either immediately or within 24 hours. Where the interval between collection and agglutination was greater than 1 to 2 hours the specimen was placed in the refrigerator. The clot was freed by rimming with wooden applicators; and the specimen was centrifuged at 2000 revolutions per minute for three minutes. Serial dilutions in physiologic saline were made immediately. The dilutions were of the customary strength, i.e., 1:10 to 1:320, except where typhus was definitely suspected, in which case the dilutions went up to 1:2560. The dilutions noted in this paper are those of sera-in-saline, before adding antigen. The final volume of diluted serum was 0.5 c.c. in each instance.

The dilutions were made in duplicate for each serum so that reactions could be obtained against the Proteus X19 and XK ("O," or nonmotile, non-flagellated strain). To these dilutions were added 0.5 c.c. of the bacterial suspensions, prepared for us by the Department of Bacteriology. The Proteus organisms were those of a stock culture obtained from the 9th Corps Area Laboratory, Monterey, California, just prior to our departure for the Southwest Pacific Area, and carried on brain heart infusion agar. As needed, growths were subcultured on the same material and washed off with formalized saline after 48 hours. The suspension was diluted to a number three strength (barium chloride standard, 1 billion organisms). When not in use, the antigen was stored in the refrigerator.

The antigen and serial dilutions were mixed by shaking, and then were placed in the water bath (37 degrees C.) for twenty-four hours. At the end of this time readings were taken in all tubes for: (1) presence of flocculent material on the bottom of the tube, (2) the clarity of the supernatant liquid. Comparison was made in each instance with 3 controls: (1) Antigen-saline; (2) known positive serum; (3) known negative serum. Only those readings were called positive wherein there were both a heavy flocculation and complete or almost complete clarity of the supernatant before shaking. No dilution was called positive where more than an estimated 25 per cent of the antigen remained in suspension. The readings were conducted at the same hour each day, against daylight by the same reader in each instance. It can be stated that variations in all of the technical procedures from venipuncture to cleaning of the tubes was held to an absolute minimum. Only two of the enlisted men participated in the handling of this series.

FINDINGS

Our results are divisible into four categories, which we have arbitrarily set up to include all of the individuals tested. The divisions were created on the basis of relative immunologic probability. As shown in the accompanying table, these were:

1. Civilians, native to this area (except where noted).

2. Patients in the United States Army hospital units, in this area. These individuals had been immunized with the typhus vaccine at various intervals

up to a year and were in the hospital with a definite or suspected diagnosis of malaria, dengue, or syphilis.

3. Men with no clinical evidence of disease (Officer candidates), who likewise had been immunized variously less than a year before testing.

4. Patients who showed titers diagnostic of Scrub typhus.

A fifth group, those who showed positive Kahns, was incorporated into the second group, when it was seen that their responses to the Weil-Felix reaction were the same.

As can be seen in the accompanying table, the civilian group, native to this area, showed absolutely no reactivity to the Proteus either OXK or OX19. It can be stated that none of these individuals admitted having had

TABLE I

TABULATION OF RESULTS

OXK	OX19	POSITIVES	CIVILIANS	OFFICER CAND.	PATIENTS
0	0		36	22	72
1:10	0		5 (Merch. Mar.)	57	109
1:10	1:10		2 (Merch. Mar.)	14	12
1:10	1:20			4	3
1:20	0		2 (Merch. Mar.)	37	50
1:20	1:10			4	4
1:40	0		1 (Merch. Mar.)	8	17
1:40	1:10				6
1:80	0				6
1:160	0	12			
1:160	1:10	7			
1:160	1:40	5			
1:160	1:160	1			
1:320	1:0	3			
1:320	1:10	2			
1:320	1:20	3			
1:640	1:80	1			
1:1280	1:40	2			

typhus and that there were no agglutinations, even partial, among them. The eleven civilians who did show reactivity to either or both Proteus strains were all Merchant Marine personnel whose contact with the disease in World ports is highly probable. In the military personnel, immunized as previously described, only 14 per cent showed a reactivity in titers 1:40 or higher (OXK). Of these 7 per cent had reactions with the OXK in dilutions of 1:160 or higher. This last group were in each instance individuals who had a clinical diagnosis of Scrub typhus. Table I will show that although the titers against OX19 rose somewhat in these patients they were still low. The remaining number reacted variously between the 1:40 and 1:160 with the OXK. The OX19 here again were low.

The 86 per cent of the military personnel whose titers were 1:20 or less in either OX19 or OXK demonstrated some very interesting subgroupings. Foremost was the observation that 20 per cent gave no evidence of immunologic

response. The men who were in the hospital for irrelevant illnesses outnumbered the healthy group beyond their ratio within the totals. The second notation and probably the most important single factor was that 60 per cent of the immunized personnel, sick or well, gave absolutely no response to the OX19, while showing a 1:10 or 1:20 response with the OXK.

No attempt shall be made to question the use of the Weil-Felix reaction to interpret immunologic status in typhus. Since the isolation of the *Bacillus proteus* from the urine of typhus patients, agglutination against it has been the only universally accepted clinical procedure for evaluation of the response to the disease. Nor can there be any reasonable doubt as to the selective reaction by the Scrub typhus group to the XK strain, and the Classical group to the X19 strain. As stated above this study set out to utilize these procedures in a mass serologic analysis. Animal protection tests would have been the only alternative method of assay, and this would have been impossible with so many sera.

On this basis, therefore, it becomes evident that in a group of young men, certainly in good health at the time of vaccination, there is a minimal Weil-Felix response to the Cox yolk sac immunization procedure against Classical typhus. This conclusion is inescapable when it is seen that over 88 per cent of the group, whether clinically healthy or suffering from irrelevant illnesses, showed no serum agglutination at all against the OX19. The presence of low titer reactions in the remaining 12 per cent and the high titer in the one individual who admitted having had Classical typhus showed definite potency of our X19 antigen. The question arises as to whether or not the initial two-dose inoculation is sufficient to create an immune response or whether a "series of kick-up" injections are needed. On the other hand, it is most perplexing to find that 80 per cent of the military personnel, ill or well, gave some reactivity against the OXK strain, a great part of them being in the 1:20 dilutions. Since the native population showed no reaction to the Proteus, this immunity may be due to the active inoculation and not subclinical contact with Scrub typhus. Here again, the efficacy of our OXK antigen was proved by the same very high titer responses among the clinically positive group of Scrub typhus patients. The probability here is that the Rickettsia used in the vaccine was that of a strain incitive to antibody response against Proteus OXK. It is understood that workers with this vaccine have recommended a stimulating dose between six months and a year following the initial series. Yet one cannot anticipate a rise to OX19 titers even with stimulation, when the overwhelming evidence indicates lack of response at this time. The interval since inoculation in these troops was in no instance over a year, in 95 per cent less than ten months and in 55 per cent about 6 months. As we observed the accumulating results, there was no reason to attempt their statistical separation on the basis of elapsed time since immunization; all time groups were quite similar.

Our second problem is perhaps of more immediate significance and the findings therewith of less controversial nature. This refers to the titer at which agglutination shall be considered positive for the diagnosis of Scrub typhus. In keeping with the accepted interpretation of findings in the Weil-Felix reaction, OXK titers of 1:160 or higher were considered diagnostic of

the disease. In every instance these patients had been clinically diagnosed as having the disease. Where the titer was clearly 1:80, there was suspicion of the disease, but we did not feel justified in making a positive diagnosis. Titers of 1:40 were not considered as belonging to the positive group. The finding in so large a group of individuals, however, definitely show that this concept of diagnostic levels must be changed. Of our military personnel, 86 per cent gave reactions of 1:20 or less, making this titer the accepted limit of normal rather than the 1:40 and certainly not the 1:80. We present, therefore, for corroboration in the hands of other workers the serologic dilutions of 1:40 as being very suspicious of Scrub typhus, and level of 1:80 diagnostic of the disease. Whether these figures will hold for Classical typhus, we do *not* feel competent to discuss.

CONCLUSIONS

1. Using the Weil-Felix reaction as an index of immunologic status, 88 per cent of a group of military personnel immunized with the Cox classical typhus vaccine gave no response against the Proteus OX19.

2. Of this same group 80 per cent showed reactivity against Proteus OXK (the Scrub typhus strain).

3. Of the OXK reactions, 86 per cent were at a titer of 1:20, 1:10, or negative. From this we have concluded that a titer of 1:40 was very suspicious and 1:80 diagnostic of Scrub typhus.

4. The possibility is discussed of the vaccine employed being of a nature to incite immunity for Scrub typhus.

5. The problem of immunity against typhus requires additional immediate study relevant to cross reactions and specific protection afforded by vaccines.

Reference to literature other than that noted in the following bibliography was impossible due to our military situation.

We wish to thank the members of the Department of Bacteriology, under Lt. James B. O'Neill, for their assistance. The technical work was entirely done by Pfc. J. S. Carson and Pvt. R. D. Tuttle of this unit.

REFERENCES

Cilento, Sir Raphael: Tropical Diseases in Australasia, Brisbane, Q., 1942, W. R. Smith and Paterson.

Gay, Frederick P., et al.: Agents of Disease and Host Resistance, Springfield, 1935, Charles C Thomas.

Virus-Rickettsia Diseases, Cambridge, 1941, Harvard University Press.

Great Britain, War Office: Medical Diseases in Tropical and Sub-Tropical Areas, London, 1941.

Stitt, E. R., and Clough, P. W.: Practical Bacteriology, Haematology, and Animal Parasitology, Philadelphia, 1938, The Blakiston Co.

A RAPID AGGLUTINATION TEST TECHNIQUE*

NATHAN NAGLE, A.B., LUCILLE SCHULZE, B.S., AND J. C. WILLETT, D.V.M.
ST. LOUIS, MO.

THE principle of shaking or agitation first applied practically by Kahn[1] in 1923 in his precipitation test for syphilis accounts to a large degree for the immediate results obtained by the Kahn test. Three minutes' shaking at a speed of 280 oscillations per minute with a stroke of 1½ inches (Kahn shaking machine) produces visible aggregations with beef heart lipoids (Kahn antigen) and syphilitic serum. Eagle[2] found that shaking shortened the time required for visible aggregation of antigen and specific antiserum. Visible aggregations were obtained with *Bacillus typhosus* and its antiserum in 4 minutes' and beef heart lipoids with syphilitic serum in 2 minutes' shaking. The speed or length of stroke of shaking is not stated.

The St. Louis Health Division Laboratories have used shaking in the agglutination test technique with bacterial antigens for the past several years. This practice was begun by C. L. Pfau, now deceased, but his data are not available. During the past two years sufficient data have been accumulated to show the usefulness of the method. The procedure developed in the rapid agglutination test technique for typhoid, tularemia, brucellosis, and other bacterial diseases is essentially adding 0.3 c.c. antigen suspension to 0.3 c.c. of serum dilutions, and agitating in a Kahn shaker for 6 minutes. A preliminary reading of the test is made and reported to the physician immediately if the titer is above 1:80. The test is then incubated for 5 hours at 37° C. in a water bath, after which time the final titer is reported.

Homologous antigens were added to positive sera in dilutions and agitated in a Kahn shaker for periods extending from 2 to 10 minutes. The maximum readings in the majority of sera were obtained after the 6-minute intervals. Table I shows the results with 20 sera with readings made at 2 minute intervals up to 10 and again after 5 and 18 hours' incubation at 37° C. Practically all of the sera developed their maximum titer after 5 hours' incubation.

One hundred and twenty-nine sera submitted for typhoid, tularemia, or brucellosis were tested with *B. typhosus* antigen, and readings were made after 6 minutes' shaking, 5 and 18 hours' incubation at 37° C. All sera submitted for an agglutination test are examined routinely for all three of these diseases. In a similar manner 131 sera were tested with *Bacillus tularensis,* 133 with *Brucella abortus* and 56 with *Bacillus proteus X19* antigens. Tables II, III, and V indicate the maximum titers of sera tested with *B. typhosus, B. tularensis,* and *B. proteus X19* antigens after 5 hours' incubation at 37° C. following the preliminary 6-minute shaking period. The incubation period for *Br. abortus* must be extended beyond 5 hours to obtain the maximum titer in a small percentage of sera as shown in Table IV.

*The St. Louis Health Division Laboratories.
Received for publication, June 8, 1943.

On a small number of known positive sera the rapid agglutination test method was compared with the technique described by the Standard Methods Committee of the American Public Health Association.[3] The essential differences between the two methods are shaking and length of incubation period. The A.P.H.A. method calls for 42 hours' incubation at 37° C. for *Br. abortus*,

TABLE I

EFFECT OF SHAKING AND INCUBATION OF ANTIGEN-SERUM MIXTURES ON TITERS OF 20 SERA

SERUM WITH HOMOLOGOUS ANTIGEN	NO.	SHAKING TIME IN MINUTES					INCUBATION AT 37°C. IN HOURS	
		2	4	6	8	10	5	18
B. typhosus	1	–	20	40	40	40	40	40
B. typhosus	2	–	20	40	40	80	80	80
B. typhosus	3	20	20	20	20	20	20	40
B. typhosus	4	20	20	40	40	40	40	40
B. typhosus	5	20	40	40	80	160	160	160
B. typhosus	6	20	40	80	80	160	160	160
B. typhosus	7	20	20	40	40	80	80	160
B. typhosus	8	40	40	80	80	80	80	80
B. typhosus	9	320	320	640	640	1280	1280	1280
B. typhosus	10	1280	1280	1280	1280	1280	1280	1280
B. tularensis	11	40	80	80	80	80	80	80
B. tularensis	12	320	320	1280	1280	1280	1280	1280
B. tularensis	13	640	1280	1280	1280	1280	1280	1280
B. tularensis	14	640	1280	1280	1280	1280	1280	1280
B. tularensis	15	640	1280	1280	1280	1280	1280	1280
Br. abortus	16	160	320	640	640	640	1280	2560
Br. abortus	17	320	1280	1280	1280	1280	2560	2560
Br. abortus	18	640	1280	1280	1280	1280	1280	1280
Br. abortus	19	1280	1280	2560	2560	2560	2560	2560
Br. abortus	20	1280	1280	5120	5120	5120	5120	5120

TABLE II

EFFECT OF SHAKING AND INCUBATION OF *B. typhosus* ANTIGEN-SERUM MIXTURES ON TITERS OF 129 SERA

NO. SPEC.	6 MINUTE SHAKING	5 HRS. INCUBATION	18 HRS. INCUBATION
108	–	–	–
3	–	20	20
7	20	40	40
2	20	80	80
2	40	40	40
2	40	80	80
2	320	640	640
2	640	640	640
1	640	5120	5120

TABLE III

EFFECT OF SHAKING AND INCUBATION OF *B. tularensis* ANTIGEN-SERUM MIXTURES ON TITERS OF 131 SERA

NO. SPEC.	6 MINUTE SHAKING	5 HRS. INCUBATION	18 HRS. INCUBATION
112	–	–	–
3	20	20	20
2	20	80	80
1	80	80	80
4	160	160	160
1	160	320	320
1	320	640	640
2	640	640	640
3	1280	1280	1280
1	2560	2560	2560
1	5120	5120	5120

18 hours at 52° C. for *B. typhosus*, and 2 hours at 37° C., and overnight at 8° C. for *B. tularensis* and *B. proteus*. Table VI shows the close agreement between the results obtained on 36 sera.

TABLE IV

EFFECT OF SHAKING AND INCUBATION OF *Br. abortus* ANTIGEN-SERUM MIXTURES ON TITERS OF 133 SERA

NO. SPEC.	6 MINUTE SHAKING	5 HRS. INCUBATION	18 HRS. INCUBATION
115	–	–	–
1	–	40	80
1	20	320	320
3	80	160	160
1	80	80	160
1	160	320	320
1	640	1280	2560
1	640	2560	2560
1	1280	2560	2560
1	1280	1280	1280
1	1280	1280	2560
2	1280	5120	5120
1	2560	5120	5120
2	5120	5120	5120
1	40,960	81,920	81,920

TABLE V

EFFECT OF SHAKING AND INCUBATION OF *B. proteus X19* ANTIGEN SERUM MIXTURES ON TITERS OF 56 SERA

NO. SPEC.	6 MINUTE SHAKING	5 HRS. INCUBATION	18 HRS. INCUBATION
31	–	–	–
2	–	20	20
1	–	40	80
4	20	20	20
4	20	40	40
4	40	40	40
2	40	80	80
4	80	80	80
2	320	320	320
2	640	1280	1280

The agglutination tests recorded in Table VI for *B. typhosus* and *Br. abortus* done by the A.P.H.A. method were also read after 6 minutes' incubation at room temperature, and again after 1, 2, and 5 hours at either 52° C. or 37° C. Visible aggregations were not observed after the 6-minute period although a small number showed aggregations in low dilutions after 1-hour incubation. The low titers obtained after 5 hours' incubation showed that long incubation periods are necessary in order to obtain the maximum titer when the antigen-serum mixtures are not shaken. Duplicate tests done by the rapid method were also read after 6 minutes' shaking and again after 1, 2, and 3 hours' incubation in the water bath at the appropriate temperature. Immediate reactions in low dilutions following the 6-minute shaking period were obtained with practically all sera recorded in Table VI. Higher titers were obtained after 3 hours' incubation, although it was necessary to incubate an additional 2 hours to obtain the maximum titer.

Antigen preparation in this laboratory follows the A.P.H.A. method very closely except that for Brucella antigen, formalin is used instead of phenol and the turbidity is 500 parts per million instead of 200 parts per million. The cul-

tures used are those distributed by the National Institute of Health including *B. typhosus* "O" and "H," *B. tularensis, Br. abortus,* and *B. proteus.* Sera submitted for Typhus or Rocky Mountain Spotted Fever are first examined with a *B. proteus X19* culture composed of both motile and nonmotile organisms made into a 0.3 per cent formolized suspension with a turbidity of 500 parts per million. Sera showing at least a 1:40 titer are repeated with live culture antigens made from *B. proteus OX19* and *OX2.* Table VI shows that the formolized and live culture antigens are equally sensitive.

TABLE VI

THE RAPID AGGLUTINATION TEST COMPARED WITH THE AMERICAN PUBLIC HEALTH ASSOCIATION METHODS ON 36 SERA

NO. SERA	RAPID METHOD	A.P.H.A. METHODS
	6 MINUTES' SHAKING 5 HOURS' INCUBATION	
Typhoid		
2	20	20
3	80	40
1	160	40
1	160	160
1	320	320
1	320	80
1	640	320
1	1,280	1,280
1	10,240	10,240
Tularemia		
1	80	80
1	1,280	640
1	1,280	320
1	1,280	1,280
1	2,560	2,560
Brucellosis		
2	1,280	1,280
2	2,560	1,280
1	5,120	2,560
1	10,240	10,240
Typhus		
1	20	20
3	20	40
1	40	40
2	80	80
2	80	40
1	80	160
1	160	160
1	1,280	1,280
1	2,560	5,120

CONCLUSIONS

The inclusion of shaking in the usual agglutination test technique enables the laboratory to secure comparatively rapid results. Six minutes' agitation in a Kahn shaking machine of the antigen-serum mixtures permits preliminary positive results to be reported at once, and following 5 hours' incubation at 37° C., the final titer can be reported.

REFERENCES

1. Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation, Baltimore, 1925, Williams and Wilkins Co.
2. Eagle, H.: Specific Agglutination and Precipitation. II Velocity of the Reactions, J. Immunol. 23: 153, 1932.
3. Diagnostic Procedures and Reagents, New York, 1941, Am. Pub. Health Assoc.

CRITERIA FOR THE SELECTION OF AN ELECTROENCEPHALOGRAPH*

LOUIS W. MAX, PH.D.,† LEO WIESNER, PH.D.,‡ AND
JESSE G. M. BULLOWA, M.D.,§
NEW YORK, N. Y.

THE history of electrophysiology has repeatedly shown that experimental results may be distorted by the physical limitations of the recording instruments. This danger is especially marked in the comparatively new field of electroencephalography, where standardization of apparatus and procedure has not kept pace with the pressure for clinical applications. Accordingly, the relative advantages and disadvantages of available electroencephalographs merit careful scrutiny, since an error in judgment at the time of procurement may result in erroneous experimental and clinical conclusions.

Physical factors determining the accuracy of instruments for physiologic work and illustrations of errors introduced by inferior recorders have already been dealt with at some length by one of us;[1] the present report applies the earlier discussion to the selection of an adequate brain wave recorder.

ESSENTIAL REQUIREMENTS

1. *Constant Frequency Response.*—Among the requirements an electroencephalograph must fulfill in order to record an accurate picture of the brain potentials is constant frequency response. This means that individual brain waves of equal voltage even though of different frequencies must appear in the record as equal in height or amplitude. If the instrument cannot record the voltage of different frequencies with equal fidelity, certain waves of clinically significant frequencies may be discriminated against and escape detection.

2. *Linearity of Voltage Response.*—Another important requirement is linearity of amplitude response. The height of the pen deflections must be directly proportional to the magnitude of the brain potentials. For satisfactory recording and proportionality, this should hold within 5 per cent for pen displacements up to 7.5 millimeters on either side of the zero line.

Testing Apparatus: The following apparatus arrangement (Fig. 1) was employed to check upon the accuracy of frequency and amplitude re-

*From the Medical Service, Dr. Oswald LaRotonda, Director, of Harlem Hospital, Department of Hospitals.

†Associate Professor of Psychology, New York University, Littauer Fellow in Pneumonia Research, Harlem Hospital, New York City, Visiting Professor of Physiology, New York University College of Dentistry.

‡Physicist, Littauer Pneumonia Research Fund, Harlem Hospital, New York City.

§Clinical Professor of Medicine, New York University College of Medicine; Visiting Physician, Harlem Hospital, New York City; Visiting Physician, Willard Parker Hospital.

From the Littauer Pneumonia Research Fund of New York University, College of Medicine. These studies received additional support from the Metropolitan Life Insurance Company, and from Mr. Bernard M. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. H. Robert Samstag.

Received for publication, June 9, 1943.

sponses of three commercial varieties of electroencephalograph. An electronic oscillator generates electrical waves of variable frequency; these waves are impressed upon a calibrated attenuator which adjusts their voltage to an appropriate value; these measured waves are, in turn, supplied simultaneously to the several channels of the electroencephalograph under test (EEG) and to an Einthoven electrocardiograph (ECG). The latter provides an independent, photographic check on the wave form, frequency, and amplitude of the test waves. The Einthoven galvanometer was adjusted to a constant frequency response from 0 to 100 cycles per second (c.p.s.).

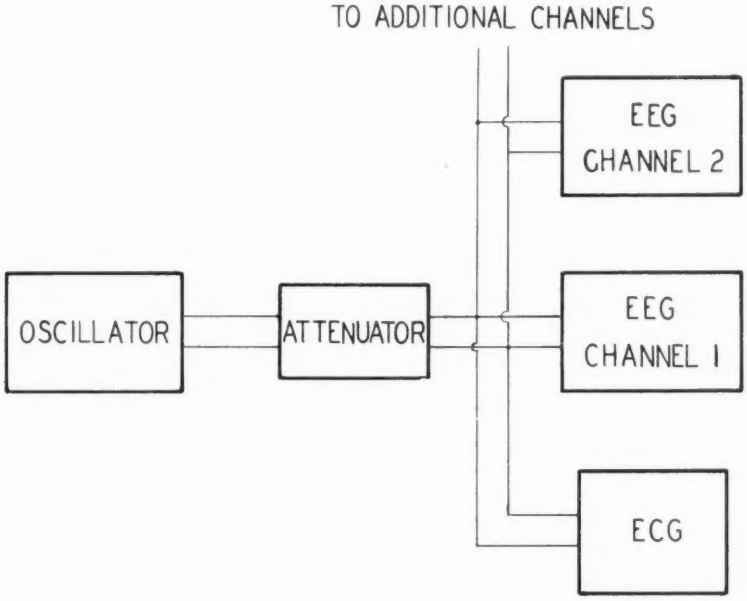

Fig. 1.

Fig. 2 presents the results of the above test on the three varieties of electroencephalographs tested. The curves present, in millimeters, the responses of the recording pens to electrical waves constant in amplitude but varying in frequency from 1 to 100 c.p.s.

It will be noted that while none of the recorders has a flat response in the entire range, the curves from two of the recorders, designated A and B, are markedly irregular in the range from 1 to 10 cycles, a range of frequencies of marked clinical importance. All of the curves are relatively flat in the range from 10 to 20 cycles, but instrument A showed an undesirable resonance peak at 25 cycles. Since this peak is well within the range of the physiologic frequencies to be recorded, serious frequency distortion would result.

In amplitude response, none of the models proved precisely linear, but the irregularities were not as important as in the case of the frequency response. Even the best of the three recorders deviated from strict linearity by a maximum of 13 per cent (± 6.5 per cent).

3. *Freedom From Temperature and Humidity Effects.*—It is important that variations of temperature and humidity encountered in the average

laboratory shall not interfere with the stability of the recorder's operating characteristics. One of the instruments tested, model B, showed varying frequency and amplitude responses on different days; these, we discovered, were a function of its proximity to a warm radiator. This model was tested on a rolling laboratory table, and its stability of response depended on its distance from the radiator. Model A was free of this temperature effect, but its writing pens failed to maintain a constant zero position on moist days. Model C remained unaffected by such temperature and humidity variations.

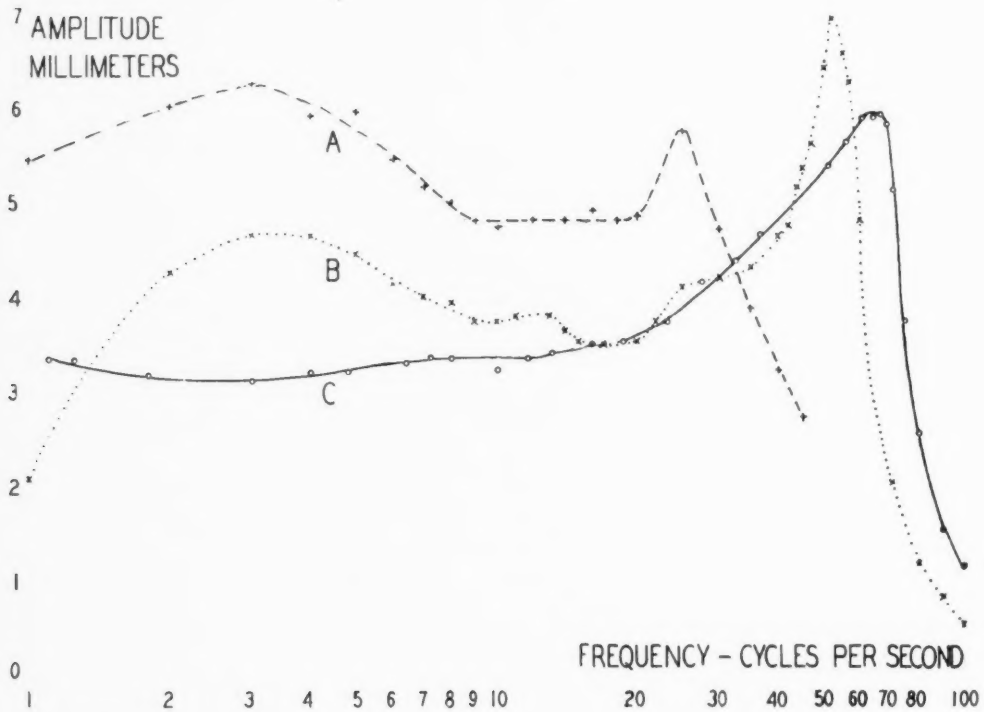

Fig. 2.

The following requirements seemed to be adequately met by all three of the models studied:

4. *Identity of Component Channels.*—Where, as is usually the case, a multiple channel electroencephalograph is to be purchased, the channels must be adjustable so that identical input voltages will produce identical records on all channels. This is important because it is sometimes desirable to switch electrode leads from one channel to another in the course of an electroencephalographic examination. Hence, a multiple channel instrument should meet the following test: After all the channels have been adjusted to give equal pen deflections for equal input voltages, all the channel inputs are to be connected in parallel to the same two electrodes on the patient's skull and simultaneous tracings taken. The tracings may then be superimposed on each other and no two of them should differ by more than ±5 per cent. If the amplifiers or recording pens are not well matched, the instrument will not pass this test.

5. *Freedom From "Cross Talk."*—One channel must not interfere with or influence the others. Such interference, termed "cross talk" by analogy with a similar disturbance in telephone and radio communication, makes the records worthless.

One way to check this is to connect one channel to the patient's skull; connect the inputs of the remaining channels to a noninductive 5000 ohm resistor and adjust them for maximum amplification. No component of the brain wave record of the first channel should appear in the other channels.

6. *Low Noise Level.*—Where no voltage is applied to the input of the electroencephalograph, one would expect a zero deflection or straight line to be recorded. Actually the line will probably contain some slight fluctuations termed "noise." Since brain waves of an amplitude lower than these random fluctuations cannot be detected, the "noise level" should be as low as possible and should not in any event exceed 2 microvolts. To check this, connect the inputs of all the channels to a noninductive 5000 ohm resistor (to simulate the patient's resistance); adjust the amplifiers for maximum gain and inscribe a record. As estimated from the microvolt calibration, the noise fluctuation should remain below the two microvolt level. This is particularly important for instruments containing B batteries, since the adventitious noise will increase as the batteries age and will later prevent satisfactory recording.

In view of the optimistic claims of manufacturers, purchase orders for an electroencephalograph should include definite specifications in accordance with the above requirements.

SUMMARY

Criteria for the adequacy of an electroencephalograph are described, and tests are suggested for checking its performance. Frequency response curves of three commercial instruments are presented.

REFERENCE

1. Max, L. W.: An Experimental Study of the Motor Theory of Consciousness I. Critique of earlier studies, J. Gen. Psychol. **11**: 112-125 (cf. especially pp. 117-122), 1934.

SOME EXPERIMENTS ON THE ROMANOVSKY STAINING OF BLOOD FILMS

R. D. Lillie, M.D.,* Bethesda, Md.

IN the course of testing samples of Wright and Giemsa stains, it became necessary to arrive at a technique which would give the best tinctorial results of which the sample was capable, regardless of time or of convenience of the method, and these experiments led to results which appear to be of general practical interest.

Controlled experiments were used, using several samples of dye, varied staining times, and varied delutions. As test objects thin blood films from white rats heavily infected with *Trypanosoma equiperdum* were used. These were fixed as soon as dry in absolute methanol for 2 minutes. A phosphate buffer of pH 6.5 was used throughout, as this gives a good orange-pink color to erythrocytes and satisfactory blue cytoplasm to lymphocytes. The stock buffer contained 6.2 Gm. KH_2PO_4 and 3.0 Gm. Na_2HPO_4 in 500 c.c. each of CP methanol and distilled water and was diluted 1:20 with distilled water for use.

In the first experiment 1/2 and 1/3 dilutions of Wright stains LWr-17 and NWr-18 were used for 1, 2, 4, 7.5, and 15 minutes; 1/5 dilution for 2, 4, 7.5, 15, and 30 minutes; 1/10 for 4, 7.5, 15, 30, and 60 minutes; 1/20 and 1/32 for 7.5, 15, 30, 60, and 120 minutes, and 1/50 for 15 and 30 minutes, 1, 2, and 4 hours. The dilutions were made in test tubes and thoroughly mixed, as this procedure was found to give more uniform staining throughout the smear than the traditional method of mixing on the slide.

The 1/2 dilution, that is, one part of methanol stain solution and one of buffered water, gave fair staining of nuclei in blue to violet tints in 4 to 15 minutes, but the desired reddish purple chromatin staining was not attained. This coloration appeared first with the 1/3 dilution in 4 to 15 minutes, best at 7.5, rather dark in 15, but was best with the 1/5 and 1/10 dilutions in 7.5 to 30 and 15 to 60 minutes respectively. Satisfactory staining was still attained with the 1/20 dilution in 1 and 2 hours, but with higher dilutions did not occur even in 4 hours. The optimal dilutions and times of those tried were 1/5 for 15 minutes and 1/10 for 30 minutes.

Next, a similar series of stains was made with two samples of Giemsa stain, NGe-13 and NIH-2B, using 6 adjacent time intervals out of the following series: 2, 4, 7.5, 15, 30 minutes, 1, 2, 4, and 6 hours, for each dilution. The dilutions used were 1/10, 1/17, 1/25, 1/36, 1/50, 1/75, and 1/100. The two samples gave fairly similar results, with a slight but consistent difference in favor of the second. Optimal staining gave deep red purple leucocyte and lymphocyte nuclei, clear medium blue or slightly greenish blue cytoplasm of lymphocytes and trypanosomes, and sharply stained trophonuclei, blepharoplasts, and un-

*Senior Surgeon, U. S. Public Health Service, Division of Pathology, National Institute of Health.

Received for publication, June 17, 1943.

dulating membranes. It was noted in this and all subsequent experiments that when trypanosome staining was optimal, lymphocytes were somewhat overstained, while with optimal lymphocyte staining undulating membranes were often indistinct.

Times required for optimal staining with the various dilutions were as follows: 1/10, 15 to 60 minutes; 1/17, 1/25, and 1/36, 30 to 60 minutes; 1/50 and 1/75, 1 to 2 hours; and 1/100, 2 to 4 hours.

Following our experience with the acceleration of azure eosinate staining of tissues with acetone,[1] it was determined to apply this method to Giemsa staining of blood films. Accordingly, eleven 40 c.c. quantities of 1:50 dilution buffered to pH 6.5 were made up, using Giemsa stain NIH-2B and containing 0 to 10 c.c. acetone by 1 c.c. steps. Five staining intervals were used with each mixture, spaced so that each successive interval was approximately twice as long as the previous one.

Good but not quite optimal staining was attained in one hour without acetone. Optimal staining was attained in one hour with 1 c.c. acetone, in 30 minutes with 2 or 3 c.c. acetone, in 15 minutes with 3 or 4 c.c. acetone; good but suboptimal staining in 30 minutes with 5 or 6 c.c. acetone; inferior staining in any interval with 7 or 8 c.c., and very poor with 9 or 10 c.c.

As 3 c.c. acetone to 40 c.c. dilute stain seemed to be about the optimal quantity, it was decided to determine the effect of various dilutions of stock Giemsa NIH-2B for various time intervals, with and without acetone.

Five dilutions were used: 0.8 c.c., 1.0, 1.2, 1.4, and 1.6 c.c. stock stain to a total of 40 c.c. diluted mixture, (ranging from 1/50 to 1/25 dilution), with and without 3 c.c. acetone in the mixture. Four time intervals were used, each double the preceding as before. Optimal staining times for the 5 dilutions without acetone were 1 to 2 hours for the first two dilutions, 30 to 60 minutes for the remaining three. With acetone, times were 30 to 60 minutes for the first 2 dilutions; 15 to 30 minutes for the next two; and 15 or less for the last. Similar results were attained on repetition of this test using Giemsa stains NIH-2B, NGe-13, and LGe-6 at 1/50 dilution without acetone and 1/25 with acetone. The acceleration of staining in favor of the latter was between 3 and 4 times.

As these tests indicated that more concentrated dilutions of Giemsa stain might give even faster staining, a further series of tests was made using 2, 2.5, 3, 3.5, and 4 c.c. Giemsa stain LGe-6, 3 c.c. acetone and water buffered to pH 6.5 to make 40 c.c. Time intervals used were 2.5, 5, 7.5, 10, 15, 20, and 30 minutes. Optimal staining of trypanosomes and blood cells was attained in 2.5 to 5 minutes with the 1/10 dilution, while about 10 to 15 minutes was needed with the weakest (1/20) dilution.

Repeating this experience on a limited number of unfixed thick and methanol-fixed thin human blood films containing *Plasmodium vivax*, stains comparable to those obtained in 30 to 60 minutes at 1:50 dilution without acetone were attained in 15 to 20 minutes at 1:25 with acetone, and in 5 minutes at 1/10 dilution. The Giemsa (N43) used in this test contained some relative surplus of methylene blue, so repetition of the test with three other samples, NIH-2A, LGe-6, and NGe-13, was done, using thick and thin films containing *Plasmodium malariae*. Again, acceptable staining of parasite chromatin was attained in 6 minutes in both thick and thin films with all three samples and with one in 4

minutes. The thick films were prepared in the morning and stained the same afternoon. It is noteworthy that of 21 thick films stained 2 to 16 minutes, none were lost, while of 4 control thick films stained 30 to 90 minutes in 1:50 Giemsa without acetone, only one had a small area left to look at on the slide.

In the course of these experiments it was noted that one series fixed with previously used methanol seemed to give slightly better staining than expected. Thinking that this might be due to the presence of some water in the used methanol, experiments were made using 100 per cent, 95 per cent, 90 per cent, 85 per cent, 80 per cent, 70 per cent, 60 per cent and 50 per cent methanol in distilled water. Two lots of Giemsa were used, NIH-2B and LGe-6, 1/50 dilution without acetone, and 1/25 with 3 c.c. acetone to 40 c.c. with the first, the latter only with the second.

The 50 per cent and 60 per cent methanol gave complete hemolysis. Much karyolysis also was evident with 50 per cent methanol, fairly good leucocyte and rather poor trypanosome staining with 60 per cent. With 70 per cent some red corpuscles were intact, others partly hemolyzed, and with 80 to 100 per cent methanol red corpuscles were well fixed. There was no appreciable difference in the minimum time required for optimal staining of lymphocytes, leucocytes, and trypanosomes with methanol percentages from 70 to 100 per cent. Prolonging the fixation interval in 90 to 100 per cent methanol from 2 to 10 minutes appeared to improve the staining somewhat.

The Giemsa stain NIH-2B, which was the one most used in these experiments and appeared to be equal to or better than the commercial samples, was made in our laboratory from methylene blue and eosin by the method recently reported.[2] The procedure is quoted as follows, since the original may not be available to hospital laboratories.

"Dissolve 10 gm. methylene blue of 85-88 percent dye content in 600 cc. distilled water. Add 6.8 cc. concentrated sulfuric acid (sp. gr. 1.835 to 1.84). Bring to a boil and add 2.5 gm. potassium bichromate dissolved in 25 cc. distilled water. Boil 20 minutes. Cool to 10° C. or lower (place in refrigerator over night). When cold add 17.5 gm. sodium bicarbonate slowly with frequent shaking. Then add a 5 percent solution of eosin Y of about 90 percent dye content and shake constantly until margin of fluid appears pale blue or bluish pink. About 205 cc. will be required and three-fourths of this can be added at once. Filter at once, preferably on vacuum funnel with hard paper. When fluid has been drawn through and surface begins to crack, add 100 cc. distilled water, let drain, and wash again with a second 100 cc. distilled water. Lay the (opened out) filter on a larger piece of filter paper or paper towel and dry overnight on warm plate or in incubator, at 37°C. The drying may be accelerated by using two 100 cc. portions of acetone or, preferably, 95 percent alcohol as washes after the second wash with water. Drying at 55° to 60° C. has been tried, and produces quite a little alteration of the thiazin dye; less if acetone or alcohol washes are used and the heating limited to 2 or 3 hours. This is the crude azure B eosinate.

"To make the crude azure A eosinate, proceed exactly as above but take 5 gm. potassium bichromate in place of 2.5 gm. and dissolve it in 50 cc. distilled water.

"To make the methylene blue eosinate, dissolve 10 gm. methylene blue in

600 cc. cold distilled water and precipitate as before with 5 percent eosin, filtering and drying as above."

"To make the finished stain, grind the three eosinates separately into fine powder in separate clean mortars. (The same mortar may be used if washed out with water, dried with a paper towel, washed with concentrated sulfuric acid until no more green color is liberated, then again with water and alcohol.) Then weigh out 500 mg. crude azure B eosinate, 100 mg. crude azure A eosinate, 400 mg. methylene blue eosinate, and 200 mg. finely ground methylene blue. Decant the mixed powder onto the surface of 200 cc. of solvent allowing it to settle in gradually. Then shake frequently for 2 or 3 days, keeping the bottle between 50° and 60° C. between shakings.

"The traditional solvent is equal parts of glycerin and methyl alcohol. If the bottle is tightly stoppered and the fluid level marked on the outside with a grease pencil or a piece of adhesive, there will be little or no loss from evaporation, and in any case the fluid level can be restored by addition of methyl alcohol. The glycerin should be neutral, anhydrous, and of the purest grade obtainable. If the special methyl alcohol for blood work is unobtainable, ordinary C.P. methonal may be repurified by distillation in glass after adding 4-5 gm. each of silver nitrate and sodium hydroxide. This destroys aldehydes and anchors volatile acids as sodium salts."

CONCLUSIONS

Wright stain solutions made up in methanol in the usual 125 mg. per 100 c.c. proportions can be made to give blood and parasite stains comparable in color effects to Giemsa by increasing the dilution to 1/5 to 1/10 and prolonging the staining interval to 15 to 30 minutes.

Previous fixation in 80 to 100 per cent methanol and staining with premixed dilutions of either Wright or Giemsa stain is the preferable procedure. This indicates that methanol may be used repeatedly for fixation, as long as no evident hemolysis is produced.

Addition of 5 to 10 per cent acetone, best 7.5 or 3 c.c. to 40, decreases the time required for Giemsa staining to about half. Increase in the concentration of Giemsa stain in the final staining mixture decreases the required time proportionately so that with a 1:10 Giemsa stain dilution containing 7.5 per cent acetone excellent stains of thin blood films are obtained in 5 minutes, and thick films may be adequately stained in 5 to 6 minutes in the same mixture. Further, thick films may be stained promptly with much less danger of losing them when such short intervals are employed.

REFERENCES

1. Lillie, R. D.: On the Use of Acetone and Methanol in Romanovsky Staining of Tissues. In press, Stain Technol.
2. Lillie, R. D.: A Giemsa Stain of Quite Constant Composition and Performance, Made in the Laboratory From Eosin and Methylene Blue, Pub. Health Repts. **58**: 449, 1943.

THE RECORDING OF LUNG CONTRACTIONS BY A PULLEY AND LEVER METHOD*

D. E. Jackson, M.D., Cincinnati, Ohio

A VARIETY of methods have been used by different investigators to study the reactions of the bronchioles to drugs, to electrical stimulation of nerves, blood pressure changes, air pressure variations, and other phenomena connected with the functions of the lungs and the respiratory air passages. The lungs and the air passages have such varied and extensive functions to perform that no single method could be expected to cover all phases of these phenomena. Earlier workers have placed a lobe of one lung in a plethysmograph (Brodie and Dixon, M. Cloetta) or have used a metal plate ("lung shield") in the chest to form a plethysmograph for one lung (Jackson), or a "lung plethysmograph" was placed inside the chest[1] to hold the walls in a relatively fixed position while the chest cavity was intermittently aspirated to carry on artificial respiration, the animal usually being pithed (Jackson). Another method involved the insertion of a cannula into the chest cavity, or the passage of a perforated tube transversely through the anterior portion of the chest wall, thus permitting air pressure changes from both pleural cavities to be recorded. More recently many workers have studied the reactions of small rings of isolated sections cut from the bronchioles. Sollmann and his co-workers have perfused isolated lungs through the trachea and noted the rate of outflow through perforations in the lungs. They have also used a special method to make microscopic cinematic studies of cross sections of fresh bronchioles.[2]

Most of these methods are too complicated or too time-consuming to be easily used by ordinary beginning medical students. Recently I have been using a method which I believe may be readily employed in ordinary class work.

Fig. 1 illustrates the apparatus and the technique which I have used. The animal (dog) is anesthetized and placed on the operating table. The chest is opened by a mid-line incision, the sternum being sawed through endwise, and four strong ligatures about eighteen inches long are passed through the edges of the opening in the chest and tied around the sawed edges of the sternum. These ligatures usually shut off the internal mammary arteries and veins and thus check any bleeding from cut branches of these vessels. At the upper end of the opening in the chest the mammary arteries should be examined and separated down to their origins, for in some animals these arteries branch off from a single stem. If this condition is found, then one of the arteries should be doubly ligated close to its origin and the artery should then be cut between the ligatures. The chest should now be pulled open about three or four inches and held in this position by tying the ligatures at the side of the dog board. If

*From the Department of Pharmacology of the University of Cincinnati College of Medicine.

Received for publication, September 9, 1943.

the chest is thus pulled open in an animal in which the mammary arteries come off from one stem, one of them may be torn off unless it has been tied and sectioned previously.

Positive artificial respiration is used, the lungs being well but not excessively inflated at the rate of about twenty-five or thirty times per minute (dog). The lungs and heart are freed from the membranous anterior mediastinum, and care is taken to see that all lobes move freely in the chest and over the heart. A string about four feet long which is as free as possible from stretch (a fishing line is very satisfactory) is now attached by a stitch to the tip of one lobe of the lungs. I have generally used the upper right lobe, but in some cases other lobes may be used to advantage.

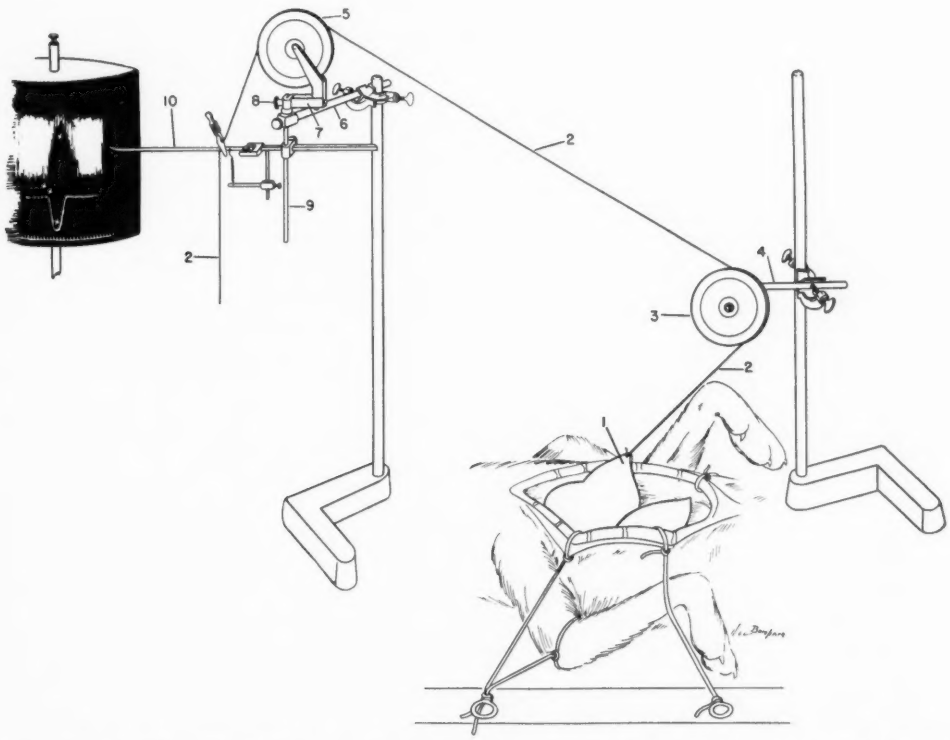

Fig. 1.—Diagrammatic representation of the lung apparatus and its method of application. For discussion, see text. (Apparatus made by the Max Wocher & Son Co., Cincinnati, Ohio.)

In Fig. 1 the string (*2*) is attached to the tip of the right upper lobe of the lung. The string passes over a pulley (*3*), four inches in diameter, and thence to the second pulley (*5*), also four inches in diameter. The distal end of the string is then clamped to the writing lever by a small, light bulldog clamp, which allows the string to be quickly and easily adjusted to give the desired magnification by the lever, or to make the lever write higher or lower on the drum. The two pulleys are made of light wood but have metal hubs which turn very freely on metal spindles. The pulleys are made large so that they can be turned very easily and thus not hamper the movements of the

lung lobe. The pulley *3* is supported by the rod *4*, which can be turned in the clamp on the stand. But the end of the rod *4* is bent at a right angle (behind the pulley *3*) and this short bent end turns in the metal block which carries the spindle supporting the pulley. This adjustment is secured by a setscrew in the metal block. By these two adjustments the pulley can be placed in any position and made to rotate in a plane which will direct the string toward the second pulley *5*, and also in such a position that the string attached to the lung can pull in the proper direction to record the movements most advantageously. The pulley *5* has some six different adjustments which are attained by the special arrangements shown in the parts *6*, *7*, *8*, and *9*. The writing lever (*10*) also has three adjustments, up and down (on the rod *9*), circularly (on the rod *9*), and back and forth (through the block and setscrew attached to rod *9*). The tension of the writing lever is partly regulated by a very fine, adjustable, coil spring and partly by the weight of the small bulldog clamp.

The writing point itself is made of thin aluminum foil so that it is light and very flexible. Since it moves at a rather rapid speed (25 to 30 double strokes per minute), it should be just stiff enough to maintain good contact with the surface of the drum. The up and down stroke on the drum should be about three and one-half to four inches (dog). It is essential that the whole mechanism works smoothly and with a minimum of friction. It is generally necessary, especially in the beginning, to do a little experimenting to find just the amount of weight and magnification to use for the lever. It can be seen that the device has a good number of adjustments. I have found that the proper adjustment is very necessary in order to get the optimum effects from the lung, and this is one of the most likely points for failure, even though the technique for the rest of the experiment has been entirely correct. At such a time I have repeatedly found that some slight readjustment of the apparatus may lead to complete success.

To obtain the best results, there will generally be two fundamental requirements which must be met. These are, first, a perfectly reliable artificial respiration machine, and, second, the animal must be kept still. Most laboratories now are equipped with respiration machines which are motor-driven and will regularly deliver constant quantities of air under constant pressure for each inspiration. An ordinary T-form of tracheal cannula is used, and the excess air delivered by the machine at each discharge is allowed to escape from the cannula through a short piece of rubber tubing which carries an adjusting (Hoffman's) screw clamp. In this manner the desired extent of lung inflation is easily secured.

Fig. 2 shows the effect which stimulation of the vagi nerves has on the lungs. Movements were recorded from the right upper lobe, but stimulation of either the right or the left vagus nerve in the cervical region caused a contraction. But the right vagus seems to be a little more effective than the left on the right lung lobe. Apparently the two vagi have so completely anastomosed before they pass into the lungs that either nerve can control either lung. This follows the embryologic development of the vagi along the gastrointestinal tract and the branching off of the lungs from the primitive gut. No doubt this is important clinically. There are some slight variations in the response of the lung to vagus stimulation, but these apparently follow very

closely the similar variations in cardiac response to the same stimulation. And the vagi endings in the lungs can be readily fatigued by stimulation just as occurs in the heart.

Fig. 2 also illustrates the marked increase in general tone of contraction of the lung lobe as shown by the whole tracing tending to rise above the normal level as the nerves are stimulated. This is also shown in another way in Fig. 4.

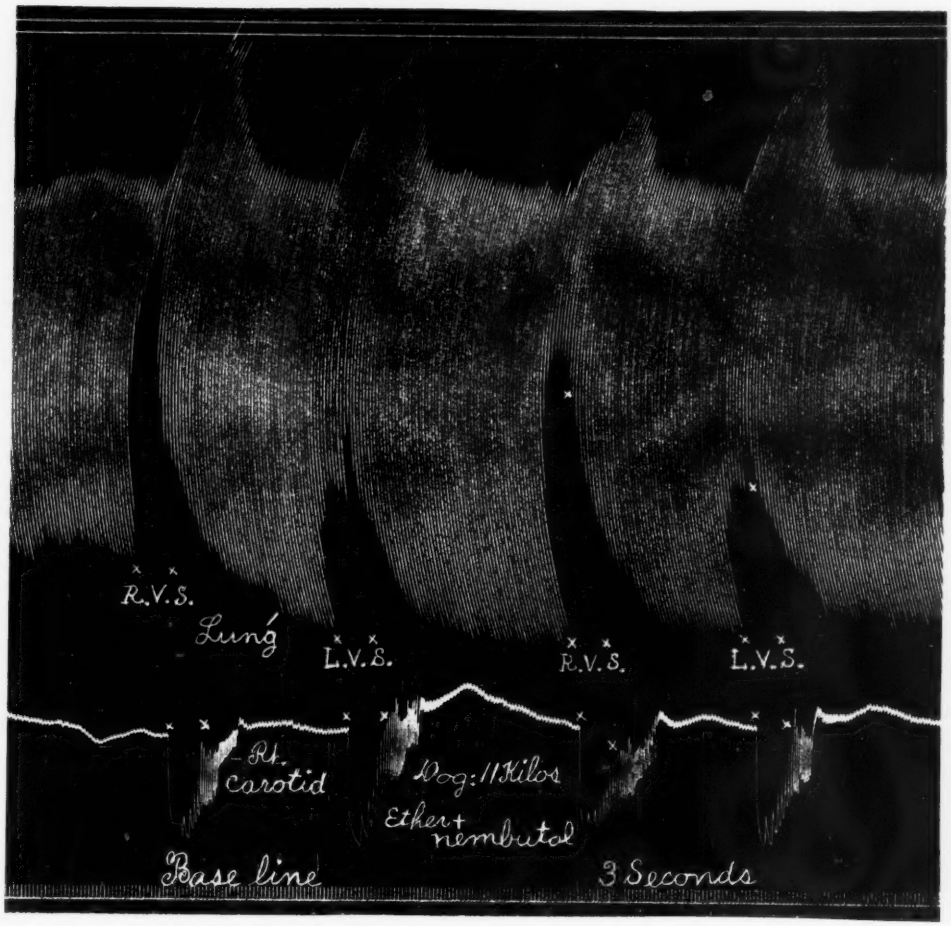

Fig. 2.—Lung tracing made from the upper lobe of the right lung, and the blood pressure recorded from the carotid artery. R. V. S., right vagus stimulated. L. V. S., left vagus stimulated.

The second basic requirement, that of keeping the animal still, may be attained in different ways. A drug of the curare type, or a large dose of nicotine, might be used. But in many cases these would be objectionable because of the ganglionic action which effective doses of these drugs may have. This might obscure or prevent entirely any central action which a drug injected later might have on the lungs. I have generally succeeded very well in keeping the animal quiet by giving ether only until all operative procedures

were completed, and then I have injected intravenously one or more cubic centimeters of nembutal solution (Abbott's solution). This is done slowly and cautiously until just the right depth of anesthesia is obtained, and then a normal tracing is taken and the drug to be studied is injected, or such other procedures as have been planned are carried out. In a certain number of instances some disturbances of the records may occur, because a profound bronchoconstriction or a great fall in blood pressure may lead to such a marked degree of asphyxia that this leads to central stimulation and may

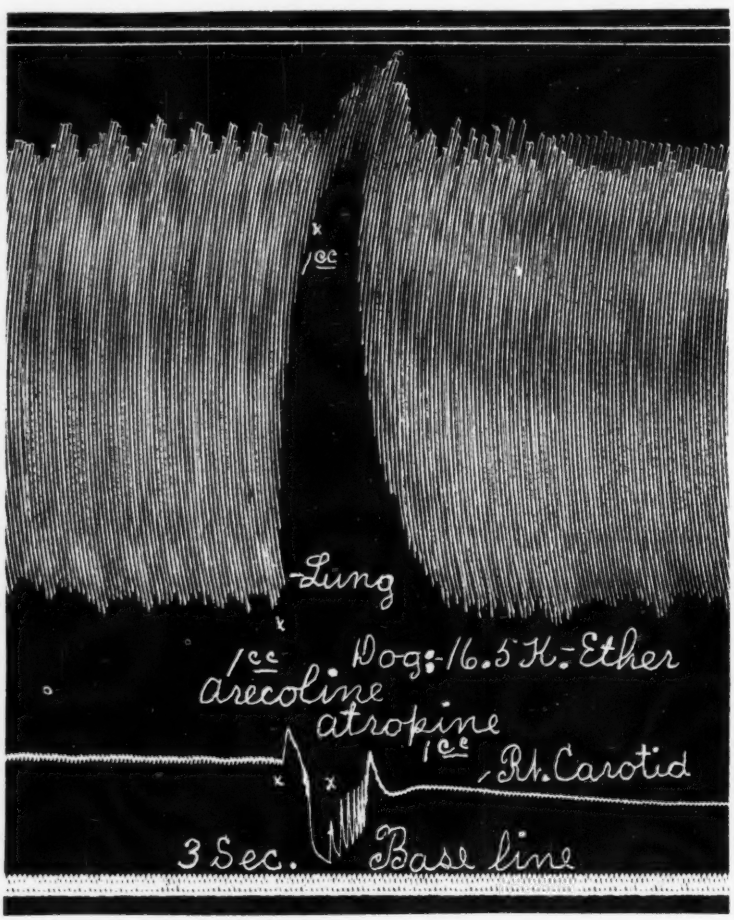

Fig. 3.—Lung tracing and blood pressure showing the action of arecoline followed by atropine. These well-known drugs are used so that the results may be compared with those obtained by other methods.

cause the animal to make special attempts to breathe deeply, or other convulsions or squirming movements may occur. A sufficient dose of nembutal may control these movements, but in some cases this may cause too great a weakness of the heart or too great a fall in the blood pressure. Such difficulties can usually be overcome by injecting smaller doses of the bronchoconstricting drugs. Possibly section of the nerves to the carotid sinuses and bodies might help in some cases.

Fig. 3 illustrates the action of two common drugs, arecoline and atropine, on the lung and on the blood pressure. There is a certain difference in form between this tracing and one made by a plethysmographic method. In the latter case the narrowest part of the lung tracing is usually near the middle of the up and down stroke of the lung tambour, and a general increase in tone of the whole lung musculature can hardly be seen in the plethysmographic tracing. But with this pulley and lever method even a slight increase in general muscular tone can be seen, even though there may be no apparent constriction of the bronchioles as this is usually interpreted in plethysmographic

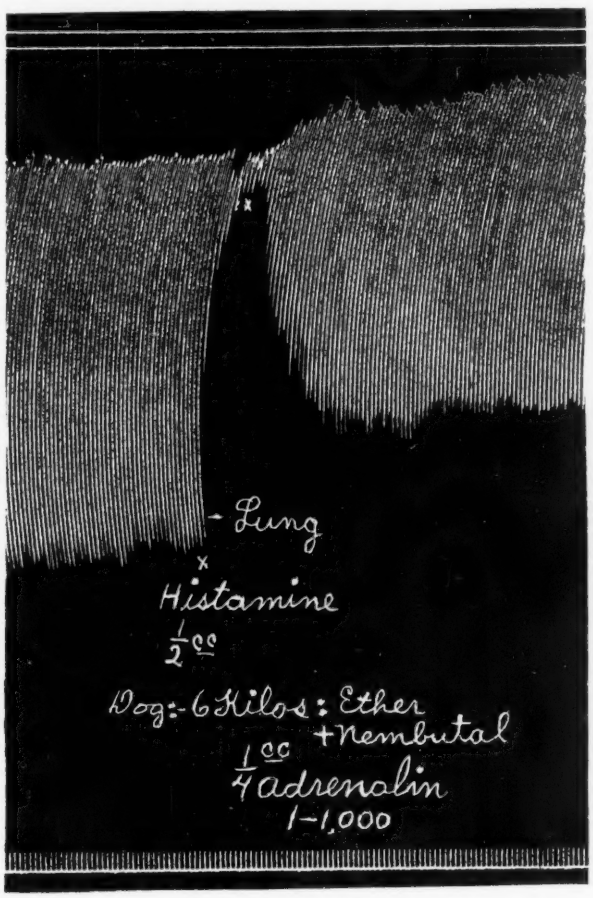

Fig. 4.—Lung tracing showing the action of histamine and adrenalin. Note increased tone (general rise of record) after histamine, even though adrenalin causes partial bronchodilatation.

tracings. A general upward shifting of the whole lung tracing as made by the pulley and lever method means an increase in tone of the whole lung musculature. Probably in the plethysmographic record the changes recorded are due mainly to alterations of the lung musculature in the transverse diameter of the lung lobes, but in the pulley and lever method the changes are obviously mainly in the longitudinal diameter of the lobe.

It can be noted that the upper limit of the left-hand portion of the lung tracing in Fig. 3 shows a kind of saw-tooth effect. This appears to be due to a peristaltic-like waxing and waning of the tone of the lung muscles. While a regular periodic variation in the force with which the air is delivered by the artificial respiration machine might produce such a variation, still as a result of many observations I believe that these effects are generally due to changes occurring within the lungs themselves, or possibly in some instances, due to changes in the pulmonary circulation brought about by variations in the efficiency of the heart.

It is advisable that the air blown into the trachea to inflate the lungs should not be introduced as a sudden strong blast. This may be avoided by passing the air through some closed vessel holding a gallon or more, just before the air is blown into the tracheal cannula. I have frequently used a five-pound ether can for this purpose. Two five-eighth inch tubulatures about one inch long were soldered into the can, one in each end, and this was placed in series with the rubber respiration tube near to the tracheal cannula. The can thus serves as a buffer to tone down a blast of air that may be too strong.

Fig. 4 shows the action of histamine and adrenalin on the lung. It will be noted that the adrenalin partly but not completely overcame the muscular constriction caused by the histamine. The persistent action of the histamine is shown by the gradual rise of the whole tracing, even after the adrenalin has produced a considerable dilatation of the bronchioles. It may be that this progressive increase in tone is due to an action on longitudinal muscle fibers running in the lung lobe more than to an effect on the circular fibers which run in the walls of the bronchioles.

SUMMARY

A simple, practical method for recording lung contractions is presented, together with some results obtained by nerve stimulation and the administration of some common drugs. This method may be useful in ordinary class experiments. It keeps the normal innervation and circulation of the lungs intact.

REFERENCES

1. Jackson, D. E.: Experimental Pharmacology and Materia Medica, ed. 2, St. Louis, 1939, The C. V. Mosby Co.
—: J. Pharmacol. & Exper. Therap. **4**: 291, 1913.
—: ibid. **5**: 479, 1914.
2. Sollmann, Torald, and Gilbert, A. J.: A Cinematic Study of Bronchiolar Reactions, Proc. Soc. Exper. Biol. & Med. **36**: 16, 18, 1937.

A NEW FORM OF MYOCARDIOGRAPH TOGETHER WITH THE TECHNIQUE NECESSARY FOR ITS USE*

D. E. Jackson, Ph.D., M.D., Cincinnati, Ohio

A VARIETY of special devices for recording contractions of the heart have been used by many investigators in the past.[1] Each of these devices has generally been designed with the object of overcoming one or more of the special difficulties involved in the nature of the experiment. Perhaps the most disturbing feature in the average experiment is the constant up and down or wavering movement which the heart assumes as a result of the inflation and deflation of the lungs by the artificial respiration machine when the chest is opened. The device shown in Fig. 1 has been designed to reduce this disturbance to a minimum. Since the device consists of two parts, the only connection between which is the string (*6*), it is easy to place each part in the most convenient position on the operating table. The writing lever and its mountings may be placed near the drum and at such an angle as will render the recording most efficient.

The experiment is performed by opening the chest of the anesthetized animal by a median longitudinal incision, the sternum being sawed through endwise, after which the chest is opened laterally about three or four inches (dog) and secured in that position by the ligatures which are tied at the sides of the operating board. Care should be taken not to rupture one of the internal mammary arteries in case both should arise from a single stem. The pericardium is opened by a median longitudinal incision, and each side of the membrane is attached up to the chest wall by a single stitch. The pericardium thus forms a kind of hammock in which the heart rests. Care must be taken to see that the heart or the incoming large veins, particularly the inferior vena cava, are not closed off or obstructed by any pull on the pericardium. It may frequently be desirable to make one or more incisions in the cut edges of the pericardium so that the heart may be permitted to beat freely and without any obstruction whatever. The stand carrying the heart levers is now brought near the edge of the operating board and so adjusted that the short levers (*2*) and (*3*) can be placed just over the heart (*1*), the levers being so oriented that their movements will be approximately in the same direction as the longitudinal axis of the heart. By means of the adjustment which is regulated by the setscrew (*5*) the levers are moved near together so that they may be sewed to the heart, each by a single stitch of a very strong thread. ("Button hole" thread is very satisfactory.) Thus the levers are left close together until the recording lever is brought up to the drum. Then, to match the other adjustments, the levers are moved farther apart again and fastened in place by the set screw (*5*). The purpose of this is to put the heart muscle on the proper tension between the attached levers. Oth-

*From the Department of Pharmacology of the University of Cincinnati College of Medicine.

Received for publication, September 16, 1943.

erwise increase or decrease of tone in the heart would not be properly shown by the writing lever.

The string (*6*) should have as little stretch as possible. (A fishing line is very satisfactory.) This string passes through the small coil spring (*9*) over the pulley (*16*), two and one-half inches in diameter, to the pulley (*17*), four inches in diameter, and finally is attached by a bulldog clamp to the writing lever. The bulldog clamp permits ready adjustment of the string so that the lever can be made to write higher or lower on the drum or the magnification can be quickly changed. The spring (*9*) serves as a flexible hollow tube through

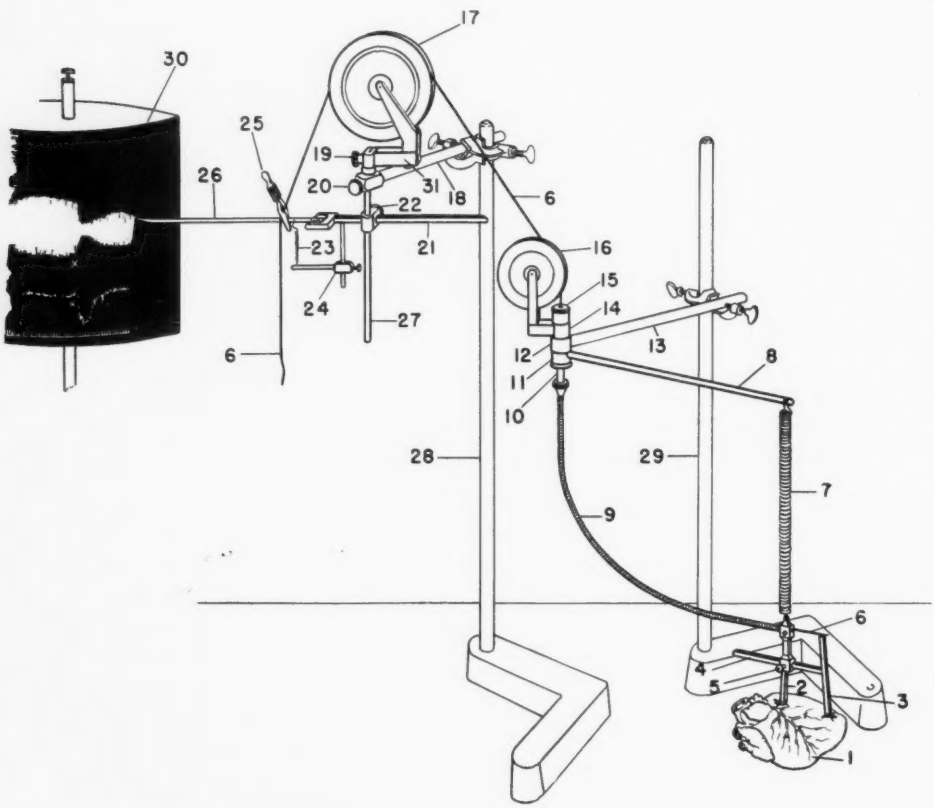

Fig. 1.—A diagrammatic representation of the myocardiograph and of its method of application. The distance between the two stands may be adjusted to suit the size and arrangement of the dog board or operating table, the position of the drum, etc., by simply extending or shortening the segment of the string *6* between the two pulleys. For discussion, see text. Device made by the Max Wocher & Son Company, Cincinnati, Ohio.

which the string slides freely. The spring is made of stiff steel or brass wire and is about one-fourth inch in diameter. The wire is wound closely, and the spring can be bent freely in any direction without changing the length of the enclosed segment of the string. It is this feature which permits the heart to be moved about by the lungs as they are inflated and deflated without these movements being recorded on the drum. The springs which I have used are sold in "dime" stores and are used to hold curtains back against window casings. The springs are cheap and very efficient.

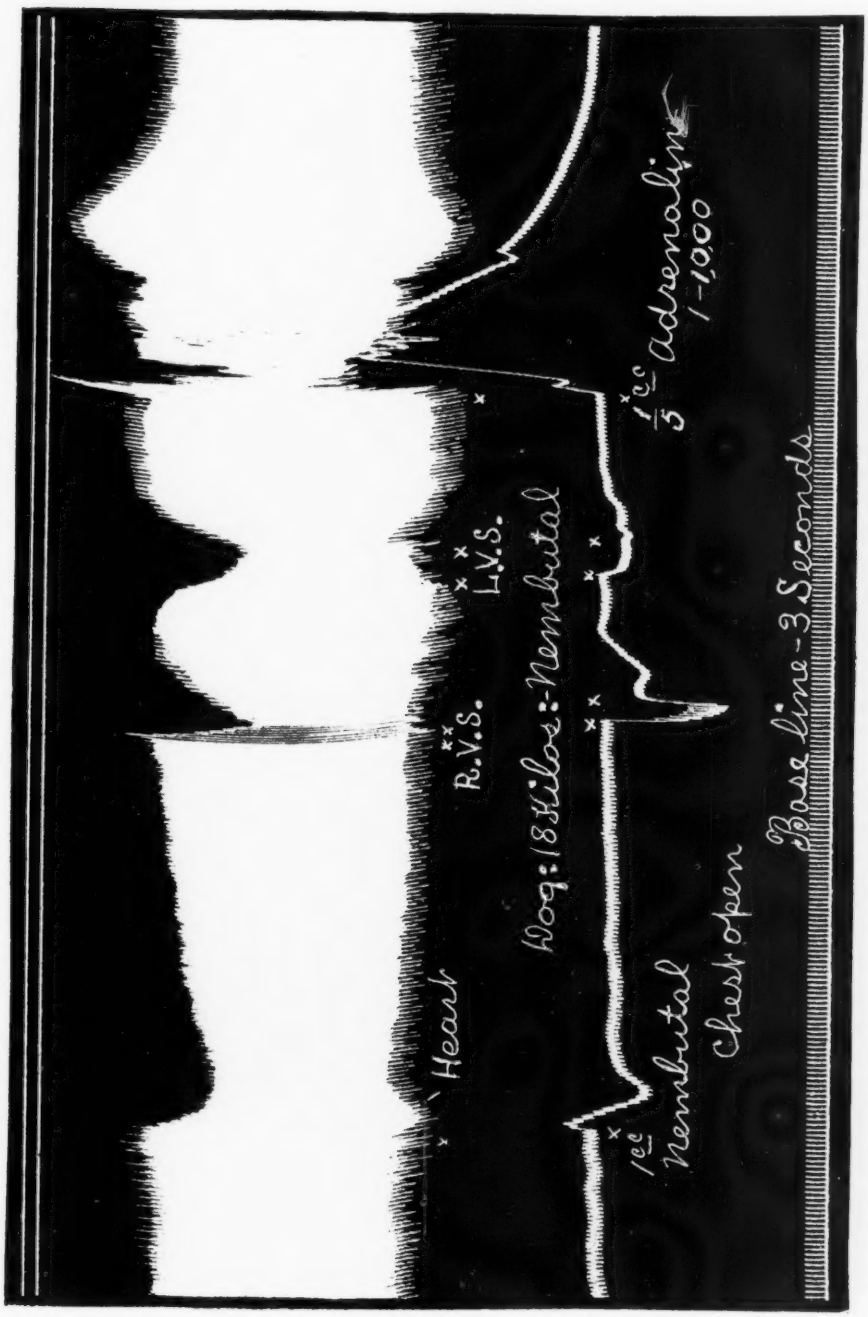

Fig. 2.—Myocardiogram and blood pressure tracing, showing the effects produced by nembutal, vagus stimulation, and injection of adrenalin. For discussion, see text.

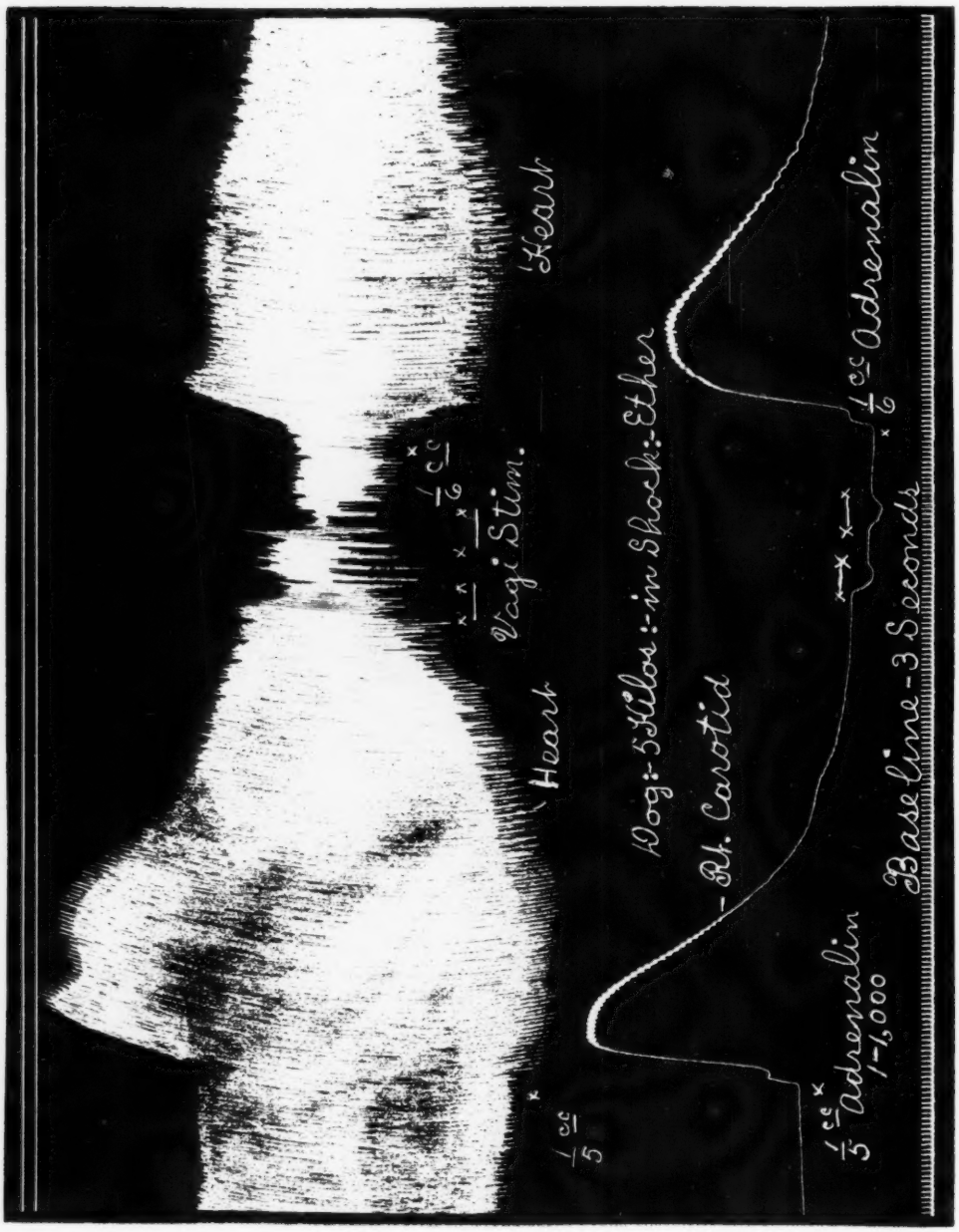

FIG. 3.—Myocardiogram and blood pressure recorded from a dog in deep shock. For discussion, see text.

The spring (*9*) is attached to the metal tube (*10*) which passes upward through the cylindrical parts *11*, *12*, and *14*. The rod (*13*) is firmly fastened to the cylinder *12*, and rod *8* is attached to cylinder *11*, while the wood pulley (two and one-half inches in diameter) is attached to cylinder *14*. All of these parts can be firmly fixed in any desired position by merely tightening the nut *15*, which is threaded on to the upper end of tube *10*. The spring *7* supports the heart levers and holds them in position just above and barely touching the heart before they are stitched to the heart. This prevents the instrument from adding any extra strain on to the heart. The pulley *16* turns very freely on its axis,

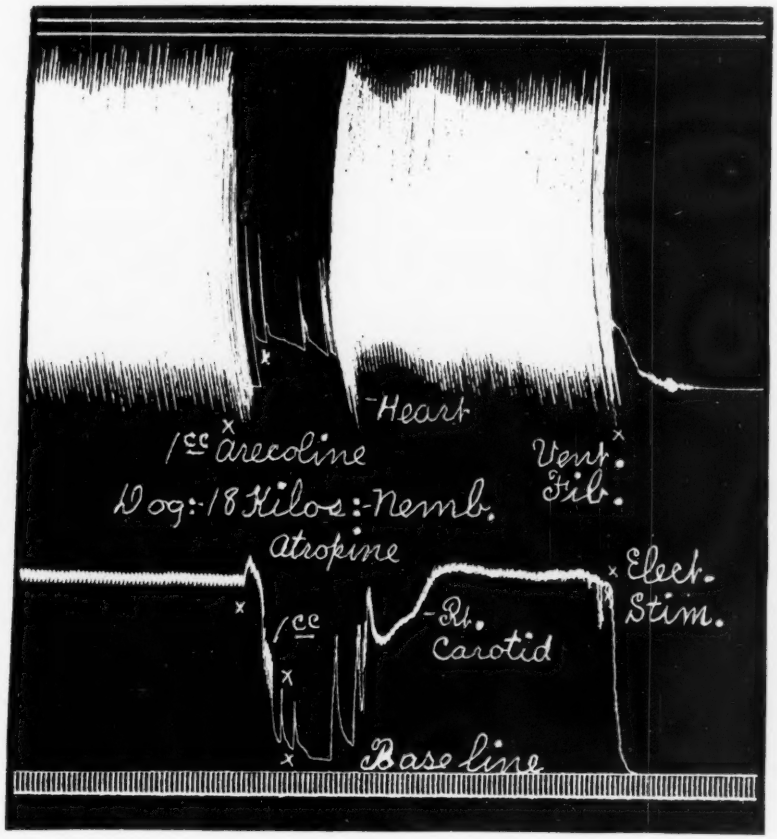

Fig. 4.—Myocardiogram and blood pressure tracing showing the action of arecoline and atropine and the development of ventricular fibrillation. For discussion, see text.

and it can be rotated on the tube *10*, so as to face the larger pulley (*17*) on the recording device. By means of the parts *18*, *19*, *20*, *27*, and *31*, the pulley *17* (made of wood) can be adjusted in six different ways. The writing lever can also be independently adjusted up and down, back and forth, or circularly around the rod *27*. Tension on the writing lever (*26*) is regulated both by the weight of the bulldog clamp and by the coil spring *23* which can be adjusted by means of the setscrew and part *24*. Positive artificial respiration must be used with this machine.

Fig. 2 illustrates some common results recorded by this device. The blood pressure was recorded from the right carotid artery. Nembutal was injected, the right and left (intact) vagi nerves were stimulated, and adrenalin was injected intravenously. The right vagus was considerably more active than the left. It will be noticed that the excursions of the heart were actually considerably reduced when it was acting against the very greatly increased blood pressure caused by the adrenalin, but as the pressure fell, the excursions increased considerably. There is always some chance that a certain amount of "fling" may be recorded by the lever when the heart is beating very fast. The lever should be light and flexible. I have found that very thin spring steel is satisfactory, but perhaps aluminum of the proper thickness and temper might be better.

Fig. 3 shows the myocardiogram and blood pressure of a dog in shock. Two injections of adrenalin were given, and the vagi nerves were stimulated. The blood pressure was so low that the vagus inhibition hardly showed in the blood pressure record, but the effect is obvious in the myocardiogram. Also the brief period during which adrenalin is effective in shock is well shown.

It should not be forgotten that experiments of this character may show a number of points which are not revealed by electrocardiographic tracings, and in many instances observations in one field might well be used to supplement information obtained in the other.

It is sometimes surprising to note how much tension or counterweight must be put on the writing lever in order to secure the optimum contraction records from the heart. The heart tracings should generally be started with an amplitude of about two and one-half to three inches, but it not infrequently happens that a slight stimulation of the heart may cause the record quickly to reach an amplitude of five or more inches, or it may become so marked that the tracings cannot well be recorded on the available drum space. In these cases the height of the initial tracing should be reduced before the stimulating procedure is started. It is highly essential that all parts of the apparatus be properly adjusted before the record is started. A certain amount of experience, and in each case some trial and error, must be carried out in order to secure a maximum of success.

Fig. 4 shows the action of arecoline and atropine on the heart and blood pressure. At the end of the tracing the heart was stimulated directly with a Faradizing current and thrown into fibrillation. The myocardiogram shows the complete diastolic state into which the heart quickly passes as the ventricles begin to fibrillate and the blood pressure promptly falls to zero.

SUMMARY

A new form of myocardiograph is described. Tracings which are made with it and which show the action of some common drugs and of some laboratory procedures are presented, together with a discussion of the technique involved.

REFERENCE

1. Several methods and instruments for recording myocardiographic tracings are given in Jackson's "Experimental Pharmacology and Materia Medica," ed. 2, St. Louis 3, Mo., 1939, C. V. Mosby Co. Most of the operative technique needed for the use of the device described in this paper is given in detail in the above text.

CHEMICAL

THE QUANTITATIVE DETERMINATION OF COMBINED CHOLESTEROL IN THE PRESENCE OF BILE*

FRANCIS F. FOLDES, M.D.,† BOSTON, MASS.

IN THE course of some experiments it became necessary to determine the eventual combined cholesterol content of the bile of different animals. In looking for a suitable method we found two methods described: one by Wright[1] and the other by Riegel and Rose.[2] The method described by Riegel and Rose appeared to be too complicated for use in a clinical laboratory. The method described by Wright proved to be unsatisfactory in our hands. By adding known quantities of cholesterol esters (acetate propionate and palmitate) alone and mixed with cholesterol to bile, the results we obtained with the Wright method were inconsistent. In the presence of bile, digitonin failed to precipitate free cholesterol quantitatively; furthermore, neither free nor combined cholesterol could be extracted quantitatively with petroleum ether from the residue of the evaporated alcohol-ether extract.

By a simple modification of the method described by Lloyd[3] for the determination of total cholesterol, we were able to determine the combined cholesterol content of bile with satisfactory accuracy. Since the bile of the animals we tested did not contain ester cholesterol, we checked the method by adding known quantities of free and combined cholesterol, dissolved in alcohol, to bile.

METHOD

Bile was diluted ten times with distilled water. To 4 c.c. of this diluted bile, 20 c.c. of 95 per cent alcohol were added in a 50 c.c. Erlenmeyer flask. After mixing thoroughly and adding a piece of quartz to avoid bumping, the mixture was heated in a water bath between 70 and 80° C. for 5 minutes. After heating, the contents of the flask were filtered through a fat-free filter into another 50 c.c. Erlenmeyer flask. The filter was washed twice with 3 c.c. of warm alcohol. Following this, the filtrate was shaken for 2 minutes with 30 c.c. of petroleum ether in a separatory funnel. After the separation of the alcohol and petroleum ether layers, the alcohol layer was allowed to flow back into the flask which had contained the filtrate. The petroleum ether phase was then poured out of the top of the funnel. The last 1 to 2 c.c. of petroleum ether in which there was a small amount of alcohol containing bile was left behind and was subsequently added to the alcohol fraction in the Erlenmeyer

*From the Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital.
†Resident in Anesthesia, Massachusetts General Hospital, Boston, Mass.
Received for publication, August 7, 1943.

flask through the stopcock. The procedure of extraction was repeated twice more. Because of the great solubility of bile salts and bile pigments in alcohol slightly (1:6) diluted with water and the equally great solubility of cholesterol in petroleum ether, after the third extraction all of the cholesterol was contained in the petroleum ether fraction and was collected in a 100 c.c. volumetric flask. All nonlipid bile constituents were contained in the greenish yellow alcohol fraction. The careful performance of the separation of the two fractions is essential for correct results, because the presence of even small amounts of bile in the petroleum ether fraction can be the cause of gross inaccuracies.

TABLE I

COMPARISON OF THE AMOUNT OF TOTAL AND COMBINED CHOLESTEROL DETERMINED AND CALCULATED

EXP. NO.		TOTAL CHOLESTEROL		COMBINED CHOLESTEROL	
		FOUND MG.	CALCULATED MG.	FOUND MG.	CALCULATED MG.
	4 c.c. palmitate solution*	0.35	---	0.22	---
	4 c.c. palmitate solution	0.34	0.35	0.23	0.22
	0.2 c.c. dog bile	0.24	---	0	---
1.	4 c.c. palmitate sol. and 0.2 c.c. dog bile	0.57	0.58	0.23	0.22
	4 c.c. palmitate sol. and 0.2 c.c. dog bile	0.57	0.58	0.21	0.22
	4 c.c. palmitate solution*	0.35	---	0.23	---
	4 c.c. palmitate solution	0.34	0.35	0.23	0.22
	0.2 c.c. cat bile	0.17	---	0	---
2.	4 c.c. palmitate sol. and 0.2 c.c. cat bile	0.50	0.52	0.22	0.23
	4 c.c. palmitate sol. and 0.2 c.c. cat bile	0.51	0.52	0.21	0.23
	0.3 c.c. acetate solution*	1.07	---	0.98	---
	0.3 c.c. acetate sol.	1.09	1.07	0.94	0.98
	0.2 c.c. dog bile	0.25	---	0	---
3.	0.3 c.c. acetate sol. and 0.2 c.c. dog bile	1.30	1.32	0.96	0.98
	0.3 c.c. acetate sol. and 0.2 c.c. dog bile	1.29	1.32	0.95	0.98
	0.3 c.c. acetate solution*	1.07	---	1.03	---
	0.3 c.c. acetate solution	1.07	1.03	1.03	1.03
	0.2 c.c. rabbit bile	0.15	---	0	---
4.	0.3 c.c. acetate sol. and 0.2 c.c. rabbit bile	1.25	1.22	1.01	1.03
	0.3 c.c. acetate sol. and 0.2 c.c. rabbit bile	1.21	1.22	1.00	1.03

*Determinations marked were made according to Bloor and Knudson's[4] method.

After the separation is completed, the extract is made up with petroleum ether to 100 c.c. Fifty c.c. of this are set aside and are used for the determination of total cholesterol as described by Lloyd.[3] The other 50 c.c., used for the determination of combined cholesterol, are placed in a 50 c.c. beaker or Erlenmeyer flask. (The volumetric flask is washed out with 1 to 2 c.c. of petroleum ether twice.) After adding a piece of quartz to avoid bumping, it is evaporated gently on a steam bath under a hood. The evaporation of petroleum ether, previous to the addition of digitonin, is necessary, because digitonin does not quantitatively precipitate cholesterol dissolved in petroleum ether. The evaporation must be slow; otherwise a large part of the cholesterol

remains on the wall of the vessel. After the evaporation of petroleum ether, 12 to 15 c.c. of hot alcohol-ether mixture (3:1) are added, allowing this to run down slowly on the wall of the vessel, thereby washing down those cholesterol particles which have precipitated on the side of the vessel. To ensure quantitative results, the vessel is now heated on a water bath covered with watch glass for 5 minutes, after which the sides are washed down with 2 to 3 c.c. of ethyl ether. This done, 2 c.c. of a 0.5 per cent digitonin solution are added, and from then on the determination proceeds exactly as given by Bloor and Knudson.[4] In the colorimetric determinations a red filter was used as recommended by Bloor.[5]

RESULTS

To check the accuracy of the method, a known amount of alcoholic solution of a cholesterol and cholesterol palmitate mixture, or a similar amount of cholesterol and cholesterol acetate, was added to bile, the cholesterol content of which had been determined beforehand. Despite the low solubility of cholesterol and cholesterol esters in alcohol, alcoholic solutions were used to ensure complete extraction by the petroleum ether. The cholesterol content of bile and cholesterol mixtures was also determined, and the values found were compared with the ones expected. The results are shown in Table I. For brevity, the cholesterol and cholesterol palmitate and cholesterol and cholesterol acetate mixtures are listed in the table as palmitate and acetate solutions respectively.

SUMMARY

A simple method is described for the simultaneous determination of total and combined cholesterol in the presence of bile.

REFERENCES

1. Wright, A.: Cholesterol and Cholesterol Esters in Dog Bile, J. Exper. Med. **59**: 407, 1934.
2. Riegel, C., and Rose, H. J.: Determination of Free and Combined Cholesterol in Bile, J. Biol. Chem. **113**: 117, 1936.
3. Lloyd, S. J.: Comparative Concentration of Human Hepatic Bilirubin and Cholesterol by the Gallbladder, Arch. Surg. **41**: 1494, 1940.
4. Bloor, W. R., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, J. Biol. Chem. **27**: 107, 1916.
5. Personal communication of Bloor to Wright; see reference No. 1.

BOOK NOTICES

Indigestion, Its Diagnosis and Management, With Special Reference to Diet*

"INDIGESTION" is a word which mystifies physicians just as frequently as medical terms mystify the layman. Rehfuss says that to the layman indigestion represents "the vast category of conditions, both inside and outside the digestive tract, that makes him conscious of his digestion." It is this fact that makes "indigestion" so vague and hard to interpret, though to the patient it may be the outstanding symptom.

Rehfuss examines indigestion from the etiologic point of view, unraveling much of the confusion caused by the unfortunate nature of the word. Sample chapter heads in his book are as follows: "Nervous Indigestion," "Indigestion due to Allergy," "Indigestion Secondary to Infection," "Indigestion due to Gastric Functional Disturbances," "Biliary Tract Type of Indigestion," "The Indigestion of Cardiovascular Disease," "The Indigestion of Old Age." Each chapter considers the diagnosis and treatment of the special type of indigestion and its underlying cause.

Over 150 pages are devoted to the special place of diet in the treatment of gastrointestinal disease. This should be especially useful to the general practitioner, students, and others as a guide for proper management of food intake, which in all disease is of major concern to the patient.

Rehfuss writes in a light, anecdotal style, drawing freely from his own experience in gastroenterology for emphasis of important points. *Indigestion* can be recommended to all as a teaching book and as a reference book for the practical management of patients with gastrointestinal complaints.

Visual Mechanisms†

VISUAL mechanisms was the subject of one of a series of symposia arranged in celebration of the Fiftieth Anniversary of the University of Chicago in 1941. Problems of vision were discussed from the physical, biochemical, physiologic, anatomic, histologic, and psychological points of view. The present volume contains additional contributions which supplement the eight original papers.

The topics include the anatomy and function of the retina and visual cells, the neurophysiology of the optic pathway, the significance of the geniculostriate system, and the originization of the occipital lobe.

There are more general discussions of cerebral organization, energy relations in vision, and a theoretic consideration of sensory acuity.

Four subjects of special interest at the present time are the photochemistry of visual purple, vitamin A, the effect of anoxia on the visual system, and alpha waves in relation to structures involved in vision.

Visual Mechanisms was written by investigators in the advance guard of visual research today. It acts in no sense as a general text in vision. Its purpose is to give an account of the possibilities of the latest research methods.

---

*Indigestion, Its Diagnosis and Management, With Special Reference to Diet. By Martin E. Rehfuss, M.D., Professor of Clinical Medicine, and Sutherland H. Prevost, Lecturer in Therapeutics, Jefferson Medical College, Philadelphia. 556 pages. W. B. Saunders Co., Philadelphia, 1943.

†Biological Symposia. Edited by Jacques Cattell. Vol. VII. Visual Mechanisms, Edited by Heinrich Klüver. The Jacques Cattell Press, Lancaster, Pa., 1942. Pp. viii + 322.

Autonomic Regulations*

THIS book is a very complete review of the nervous and hormonal mechanism which maintains the constancy of the internal environment against the stresses upon the body from the outside. Emphasis is placed on the mutual relationships of the various organs and how changes in the external environment affect the various organ systems of the body. Adjustment reactions of the respiratory and circulatory systems to carbon dioxide, anoxia, asphyxia, hemorrhage, and hypoglycemia receive attention in the first section of the book. The interrelation of the autonomic nervous system with the endocrine system receives attention, with a thorough review of the literature on the nervous regulation of the hormones of the hypothesis and of the sympathetic, adrenal, and vagoinsulin systems. The integration of the autonomic and somatic nervous systems and somatic reactions to hypoglycemia, asphyxia, and anoxia, etc., makes up the third main portion of the book. The author concludes the book with a summary of the results of the physiologic investigations of autonomic reactions and the application of this material to clinical medicine, neurology, and neuropsychiatry. In all, the book is 300 odd pages long.

There has been need for this work for some time. It is a thorough integration of knowledge which heretofore has been spread through many journals over a number of years. It will be a valuable teaching aid for students of physiology and a source book for physiologists and clinicians with a physiologic approach to disease. For the new and growing field of psychosomatic medicine, *Autonomic Regulations* will serve as a guide and orientor to a physiologic approach, which is so important for the proper development in this young branch of Internal Medicine.

Dr. Gellhorn should be praised for his bibliography, which consists of eleven hundred references to the literature of the autonomic and endocrine systems and related subjects.

Biochemistry of the Skin†

MEDICAL students and clinicians would undoubtedly welcome a satisfactory volume dealing with the chemistry of the skin and changes which occur in disease. Dr. Markowitz' book completely fails to meet the need for such a study. The work is sprinkled with erroneous statements and misspelled words. The bibliography is poorly compiled. The inclusion of such subjects as basal metabolism and racial differences in blood, among others, seems hardly justifiable, while, on the other hand, the important and related subject of the chemistry of sweat is only briefly considered. The chapters on the vitamins and dermatoses contain noncritical discussion of subjects such as achromotrichia, the use of massive doses of vitamin D for psoriasis, and the relation of biotin to cancer. References to the author's clinical experience are few and superficial. The reviewer is left puzzled concerning the value the author attaches to the Caspari diet for malignancy, first described in 1929 and included in the appendix of this book.

Polarography‡

SINCE 1922 there has developed a new theory and practice for rapid qualitative and quantitative determinations of most metals and nonmetals as well as of many organic compounds. The method is considered of value in serologic examination for the detection of sulfhydryl containing compounds, unsaturated acids, carbonyl compounds, certain nitrogen

*Autonomic Regulations. Their Significance for Physiology, Psychology and Neuropsychiatry. By Ernst Gellhorn, M.D., Ph.D., Professor of Physiology, College of Medicine, University of Illinois. With 80 Illustrations and Frontispiece. Cloth, $5.50, 375 pages. Interscience Publishers, Inc., New York, 1943.

†Practical Survey of Chemistry and Metabolism of the Skin. By Morris Markowitz, M.D. Cloth, 196 pages, $3.50. The Blakiston Company, Philadelphia, 1942.

‡Polarography, Polarographic Analysis, Voltammetry and Amperometric Titrations. By I. M. Kolthoff, Professor and Head of Division of Analytical Chemistry, University of Minnesota, Minneapolis, Minn., and James J. Lingane, Instructor in Chemistry, University of California, Berkeley, California. First Edition with 141 illustrations, 510 pages, $6.00. The Appendix contains tables of the Potentials of Common Reference Electrodes and of Half-Wave Potentials of Inorganic Substances. Eight pages can be bought separately, $0.75. Interscience Publishers, Inc., New York, 1941.

derivatives, and in making other biochemical assays. Any substance which can be electroreduced or electrooxidized can be detected and measured. The most desirable concentrations for analysis range between 10^{-4} and 10^{-2} molar, which on the average can be measured with a deviation of 2 per cent.

The analytic results of this new method depend on the interpretation of graphical current-voltage curves; hence the name "voltammetry" seems appropriate, but its founder Jaroslav Heyrovsky has called it *Polarography*. The decomposition potential of a given substance is characteristic of the particular electroreducible or electrooxidizable substance present. The "half-wave potential" is even more characteristic. The latter is recognized as the potential of the electrode in the solution of unknown against an external reference electrode, indicated by the point on a current-voltage curve where the current is equal to one-half of its limiting value. Under best conditions the limiting current is directly proportional to the concentration of the electroreducible substance. Thus, the "half-wave potential" shows the qualitative composition, while the limiting current measures quantitatively the unknown present, the interpretations being made by referring to curves of known substances. Even mixtures can often be assayed.

The authors have written a treatise, theoretical and practical, which compares with their usual high quality work. They have not only digested the information in all of the four hundred odd papers published on the subject up to 1940, but have supplied much information specifically by their own investigations. Due credit has been given to other workers, and sufficient bibliography has been supplied to aid those desiring more complete information. This book of 500 pages, the only important one written in English on Polarography, should be possessed by all interested in employing the methods and by those studying or teaching the subject.

Spectrophotometry in Medicine*

SPECTROPHOTOMETRIC methods are becoming of more and more importance in laboratory medicine; they are especially valuable in research work. This translation of Ludwig Heilmeyer's "Medizinische Spektrophotometrie" by A. Jordan and T. L. Tippell will be found of great value to any one interested either in the theory of spectrophotometry or its clinical application. Since this is primarily a translation of the original German version published in 1932, few references are made to methods developed since that time. The description of instruments, however, has been supplemented so as to bring it up-to-date. The subject matter is divided into 6 topics: (1) theory and selected methods of spectrophotometry; (2) the spectrophotometry of whole blood, of hemoglobin, and of the most important hemoglobin derivatives; (3) the first breakdown products of hemoglobin; (4) the spectrophotometry of blood serum and of normal and pathologic serum pigments; (5) the spectrophotometry of urine, and of the most important normal and pathologic urinary pigments; and (6) the spectrophotometry of bile, ascitic fluid, and cerebrospinal fluid.

*Translation of Ludwig Heilmeyer's "Medizinische Spektrophotometrie" by A. Jordan, M.B., B.S. (Lond.), D.Sc. (Strasbourg), M.R.C.P. (Lond.) Ingénieur-Chimiste E. C. M., Assistant-Pathologist E.M.S.; Junior Demonstrator in Chemical Pathology to St. Bartholomew's Hospital and T. L. Tippell. Cloth, 250 pages, $8.75, Adam Hilger Ltd., London, Eng., 1943. Obtainable in U. S. A. from The Jarrell-Ash Company, 165 Newbury Street, Boston, Mass.

Index photographed at the
beginning for the convenience
of the microfilm user.